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The  
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ANNUAL  
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ANNUAL  
REPORT

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1978



# *National Eye Institute* *Report of program activities.*

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ANNUAL REPORT  
NATIONAL EYE INSTITUTE  
October 1, 1977 - September 30, 1978

STATEMENT OF THE INSTITUTE DIRECTOR

The National Eye Institute (NEI) will soon complete its ninth year. During FY 1978, the Institute's budget grew to \$85 million, an increase of 33 percent over the previous year and almost four times the first budget for the newly-created NEI in FY 1970. The number of projects supported by the extramural portion of this budget has grown from 353 in FY 1970 to 886 this year. These funds support research directed toward reducing the physical and economic hardships caused by blindness and visual impairment by improving the ability to prevent, diagnose, and treat blinding and disabling eye disorders.

One of the most important events of the past year was the completion and publication of the National Advisory Eye Council's second major planning report, Vision Research—A National Plan: 1978-1982. This three-volume analysis of current needs and opportunities in vision research is the culmination of a two-year effort by the Council, aided by over 160 outstanding members of the vision research community. The Council and the NEI hope that this five-year plan will serve as a guide in encouraging and supporting research throughout the United States and abroad on blinding and disabling eye diseases. The document outlines the need for intensified fundamental and clinical investigation in specific areas of vision research and addresses important scientific and management issues relevant to all vision research.

Another significant development of the past year has been the NEI's increasing interest in worldwide efforts to prevent blindness. Several unique research opportunities have been identified in Latin America and elsewhere abroad, and plans are underway to initiate studies concerned with the role of nutrition in keratomalacia and xerophthalmia, leading causes of blindness in developing nations.

In the important area of clinical trials, a second report of findings from the Diabetic Retinopathy Study published in January presented new data which show that photocoagulation can reduce the rate of development of severe visual loss and inhibit the progression of retinopathy to some degree in all stages of the disease which were included in the Study. Furthermore, the data show that the original evidence of beneficial treatment effects first reported in 1976 has become even more convincing with additional follow-up.

Although the DRS has conclusively demonstrated the value of photocoagulation therapy during the later stages of diabetic retinopathy when the disease has progressed to a moderate or severe proliferative stage, the Study's findings did not provide the basis for a clear choice between prompt treatment or deferred treatment in early proliferative or severe nonproliferative retinopathy. Therefore, a new trial was initiated this year whose

principal goal is to determine the optimal time to initiate photocoagulation treatment in diabetic retinopathy. The Early Treatment of Diabetic Retinopathy Study (ETDRS) will also investigate whether treatment of early stages of diabetic retinopathy by aspirin and/or dipyridamole and/or prompt photocoagulation is effective in decreasing the rate of development of severe visual loss when compared to placebo or deferred photocoagulation. Completion of the Study design and manual of operations is scheduled for November 1978; recruitment of patients is scheduled to begin in spring 1979. The ETDRS is further evidence of the NEI's strong interest in helping develop the best possible program of care for those with ocular complications of diabetes.

Heightened interest within the research community in clinical trials is evidenced by the increasing number of grant applications being received for the support of such studies. As a result, the Institute is now supporting a number of investigator-initiated clinical trials under research grants. Procedures for reviewing and awarding research project grants for investigator-initiated clinical trials were augmented during the past year. Successful applicants are now first awarded a grant for developing and testing a manual of operations that sets forth the procedures for conduct of the proposed trial. Once this requirement has been fulfilled, the funds needed for executing the study will be determined and made available via the original grant in accordance with normal NIH procedures.

Altogether, 38 departments of ophthalmology participated in NEI-supported trials during FY 1978. Seventeen of these departments participated in more than one NEI-sponsored clinical trial during that year; however, the principal investigators were different in almost all instances. This involvement of more than one-third of the departments of ophthalmology in the United States in controlled clinical trials supported by the NEI is one indication of the influence of the example of the Diabetic Retinopathy Study as well as workshops, courses, and publications on the use of clinical trials in vision research.

Examples of new NEI grant-supported clinical trials in various stages of development are an evaluation of the effect of vitamin E on prevention of retrolental fibroplasia, the effect of photocoagulation in shortening the duration of central serous chorioretinopathy, the effect of photocoagulation in the treatment of senile macular degeneration, the effect of photocoagulation on the treatment of retinal branch vein occlusion, and the safety and efficacy of orthokeratology in changing corneal curvature to reduce refractive error. The fact that the latter study is being performed at a school of optometry indicates increasing interest and expanding participation in clinical trials from this segment of the vision research community as well.

Highlights of accomplishments in other areas of science base and applied research may be found in the following reports of NEI extramural and intramural programs.

As the National Eye Institute approaches its tenth anniversary, it is gratifying to look back upon a decade of support for quality laboratory and

clinical research which has led to important advances against major blinding and disabling eye diseases. As a result of the strong research base established in these years, the decade to come offers hope for even greater achievements in this field.

A handwritten signature in dark ink, appearing to read 'Carl Kupfer', with a stylized, cursive script. The signature is written over the printed name below it.

Carl Kupfer, M.D.



EXTRAMURAL AND COLLABORATIVE PROGRAMS



ANNUAL REPORT  
NATIONAL EYE INSTITUTE  
October 1, 1977 - September 30, 1978

REPORT OF THE ASSOCIATE DIRECTOR FOR EXTRAMURAL AND COLLABORATIVE PROGRAMS  
Ronald G. Geller, Ph.D., Acting

Fiscal Year 1978 was a time of exciting growth and change for the Extramural and Collaborative Programs of the National Eye Institute. In keeping with the Institute's first priority, a record number (772) of grant awards for investigator-initiated individual research projects (ROI) were made covering a wealth of scientifically exciting ideas relevant to the prevention, treatment, and cure of diseases and disabilities of the visual system. Moreover, there was a marked expansion of the Institute's efforts to evaluate new treatments for diabetic retinopathy and other disorders through the medium of controlled, randomized, clinical trials and considerable progress was made in refining a set of policies and procedures for center grants and research training awards which can facilitate the rapid qualitative and quantitative maturation of vision research. The following sections highlight some of the issues and accomplishments in the NEI Extramural and Collaborative Programs during FY 1978, as well as identify opportunities for future initiatives.

For FY 1978 the National Eye Institute received an appropriation of \$85,392,000—an increase of \$21,392,000 over the previous year's appropriation. Of the \$85,392,000, a total of \$74,373,000 was allocated to Extramural and Collaborative Program activities in the following categories:

Research Grants	\$64,730,000
Research Training Awards	4,643,000
Research Contracts	<u>5,000,000</u>
Total	\$74,373,000

This funding level enabled the Institute to sustain the rapid but disciplined growth that its programs have exhibited over the past several years.

The bulk of the budget increase occurred in funds for research grants; an additional \$18,000,000 was available in this category in FY 1978 as compared to FY 1977. These funds were distributed among the Institute's five programs as follows:

	Research Dollars (in thousands)		
	FY 77	FY 78	% Growth
Retinal and Choroidal Diseases	\$17,668	\$24,733	41
Corneal Diseases	7,595	8,888	16
Cataract	4,567	6,604	45
Glaucoma	6,125	8,073	30
Sensory and Motor Disorders of Vision and Rehabilitation	<u>10,069</u>	<u>16,432</u>	<u>64</u>
Total	\$46,024	\$64,730	39

START → The grant application receipt rate was 1 1/3 times that in FY 1977, which continued to increase the workload within the Institute and throughout the review system. The National Advisory Eye Council approval rate, however, was stable during these two fiscal years: 83 percent of grants submitted were approved for funding in both FY 1977 and 1978. The Institute was able to fund 68 percent of all approved applications, an increase over FY 1977. The data are given below.

Grant Application Rate

	<u>Received &amp; Reviewed</u>	<u>Recommended for Approval</u>	<u>Approved &amp; Funded</u>	<u>% Funded of all Approved Applications</u>
FY 1977	512	425	225	53
FY 1978	673	552	375	68
% Change	+30	+30	+70	

The distribution of awards between competing and noncompeting research grant applications was as follows:

	<u>FY 1977 Number of Grants</u>	<u>FY 1978 Number of Grants</u>
Prior Year Commitments	453	506
New Research Awards	96	272
Renewal Awards	<u>129</u>	<u>108</u>
	678	886

Once the prior year commitments were taken into account, there was approximately \$26 million available for new and competing research grants--the largest amount of "new" money for investigator-initiated vision research ever available in one year in the history of the National Eye Institute. The 889 grant awards represent 2 1/2 times the number awarded in FY 1970, the first year of the National Eye Institute's existence.

The Institute's research grants are comprised of the following categories:

FY 1978 Research Grants by Mechanism  
(Dollars in Thousands)

	<u>Number</u>	<u>Total Awarded</u>
Project Grants (R01, R10, R13)	783	\$57,734
Special Visual Science		
Research Awards (R23)	8	95
Core Center Grants (P30)	19	3,003
Specialized Clinical Research		
Center Grants (P50).	7	1,523
Research Career Development Awards (K04)	55	1,946
Academic Investigator Awards (K07)	<u>14</u>	<u>429</u>
Total	886	\$64,730

The codes in parenthesis in the above table are the symbols used by NIH to differentiate the various types of grant awards. A description of each of these mechanisms can be found in the Introduction to Volume Three of the publication Vision Research--A National Plan, 1978-1982 (DHEW Publication No. (NIH) 78-1260). It is noteworthy that approximately 88 percent of FY 1978 grant funds are allocated to individual investigator-initiated research projects.

The National Eye Institute complements its research grants with a program of institutional and individual fellowships. The purpose of the program is to equip young investigators with the skills, experiences and insights necessary for them to embark successfully on a career in vision science, especially its clinical aspects, and other disciplines, such as the basic medical sciences, epidemiology, engineering and biomathematics.

A total of \$4,643,000 was available for support of vision research training in FY 1978, most of it for the National Research Service Awards (NRSA). The individual NRSA fellowship awards accounted for 32% (\$1,495,000) of available training funds. The institutional NRSA training awards accounted for \$3,148,000 or 68% of the program.

The National Eye Institute's collaborative research activities, funded through contracts, continue to emphasize cooperative clinical trials for the treatment of diabetic retinopathy. The distribution of contract awards and funds is as follows:

	<u>Number</u>	<u>Total Awarded (in thousands)</u>
Diabetic Retinopathy Study	17	\$1,878
Diabetic Retinopathy Vitrectomy Study	15	1,262
Early Treatment Diabetic Retinopathy Study	9	1,372
Other	<u>4</u>	<u>488</u>
Total	45	\$5,000

The qualitative and quantitative growth in the NEI Extramural and Collaborative Programs during the past year were accompanied by several staff and organizational changes:

1. Dr. Ronald G. Geller has joined the NEI to assume the position of Associate Director, Extramural and Collaborative Programs. Dr. Geller replaced Dr. William F. Raub who became Associate Director for Extramural Research and Training, NIH. Dr. Catherine Henley joined the NEI to assume the position of Review and Special Projects Officer. She will serve as Executive Secretary of the Vision Research Program Committee and coordinate the initial review of center grant applications, academic investigator awards, and institutional research fellowships. Dr. Israel A. Goldberg, the former Review and Special Projects Officer, has joined the Retina and Choroidal Diseases Program, Scientific Programs Branch.

2. Several personnel changes and assignments have occurred in the Scientific Programs Branch. Dr. Anita Suran joined the NEI to become the Extramural Program Director for Glaucoma. Dr. Ralph Helmsen has now assumed the position of Program Director for Corneal Diseases. Dr. C. James Bailey has joined the NEI to become the Program Director for the Sensory and Motor Disorders of Vision and Rehabilitation Program. Dr. George Steinberg has joined the NEI to become the Extramural Program Director for Cataract. He replaces Dr. Herbert Yellin who has left NEI to become Executive Secretary of the Neurological Disorders Program Project Review Committee B, NINCDS. Dr. Thelma N. Fisher has also left NEI to become Executive Secretary of the Microbiology and Infectious Diseases Advisory Committee, NIAID.
3. Several additions have been made to our staff of the Extramural Services Branch. Mr. Robert Hudson has joined the Grants Management Section as a grants management specialist. He will have major responsibility for the Sensory and Motor Disorders of Vision and Rehabilitation Program. Ms. Helen MacLellan has left the NEI's Intramural Clinical Branch to become a technical information specialist in the Program Information Section.

# VISION RESEARCH TRAINING

The National Academy of Sciences has been given by Congress the continuing responsibility to study the personnel needs of biomedical and behavioral research and its recommendations will serve as guidelines for the implementation of the National Research Act of 1974. Vision research training is an essential component of the national policy of fostering improved prevention, diagnosis, and treatment of blinding and disabling eye diseases. The training policies of the National Eye Institute have been in accord with and in fact have anticipated the recommendations of the Academy. The Academy, like the National Eye Institute, emphasizes the need to sustain the quality of the research environment to improve the chances that efforts will be directed toward research that will result in advances against disease and disability. As a result of the National Eye Institute training programs, departments of ophthalmology, schools and colleges of optometry, and other academic centers throughout the United States have developed or strengthened their research components so that multidisciplined groups with active programs now exist.

The primary mechanism for support of vision research training is the National Research Service Awards (NRSA) for individual and institutional fellowships. The present status of the NEI's research training activities is summarized in the following table:

Table I

## SUMMARY OF VISION RESEARCH TRAINING <sup>1/</sup>

	<u>Active Programs</u>	<u>Total Amount (thousands)</u>	<u>% Training Budget</u>
NRSA Individual (F32)	115 <sup>2/</sup>	\$ 1,495	32%
NRSA Institutional (T32)	43 <sup>3/ 4/</sup>	\$ 3,148	68%

1/ Total obligations for research training in FY 1978 = \$4,643,000. In addition to NRSA's, this includes \$519,000 for six Graduate Research Training Grants (T01). All Graduate Research Training Grants will be phased out by June 1979.

2/ Includes 41 new F32's.

3/ Includes one new T32 and one competing supplement.

4/ 23 currently active T32's were formerly Graduate Research Training Grants.

From this table it should be noted that approximately 32% of NEI's research training dollars are for NRSA individual fellowships (F32) and 68% for NRSA institutional training programs (T32). This is in accord with the National Academy of Sciences recommendations for distribution of research training dollars. When the information in this table is compared with the information provided by last year's Annual Report, only 41 new individual fellowships (F32)

and 1 new institutional training program (T32) were awarded in FY 1978, whereas 52 individual fellowships (F32) and 9 new institutional training programs (T32) were awarded in FY 1977. There are two major reasons for this reduction in the number of fellows and training programs supported by NEI research training funds in FY 1978: (1) the NEI research training budget has remained essentially at the same level (\$4.6 million) since FY 1974, and (2) the amount of funds available for competing individual and institutional awards for FY 1978 was reduced by the increased number of commitments made in FY 1977. With the phasing out of the Graduate Research Training Programs (T01) in June of 1979, prospects for an increased competing base and subsequent increased funding for institutional training programs and individual fellowships in FY 1979 are encouraging.

A more detailed analysis of the distribution of vision research training resources by program is provided in the table on the following page, (Table 2). The continuing objective of the National Eye Institute is to support research training relative to its five programs: Retinal and Choroidal Diseases, Corneal Diseases, Cataract, Glaucoma, and Sensory and Motor Disorders of Vision. Research training is encouraged in all the medical sciences disciplines as they apply to these five National Eye Institute programs. Foremost among these in terms of needs perceived by the National Advisory Eye Council and of opportunities in vision research are: immunology, genetics, pharmacology, epidemiology, physiology, biostatistics, biochemistry, developmental biology, psychophysics, physiologic optics, and experimental and clinical pathology. With the exception of the Sensory and Motor Disorders of Vision program, the level of training support generally parallels the research grant support (R,P,K) for a particular program (footnote 2). This suggests that the state of knowledge for the long-term advances in vision research depends upon the fundamental disciplines such as biochemistry, physiology, immunology, and anatomy.

In order to provide additional visibility to the multidisciplinary institutional research training programs supported by the National Eye Institute in the programs identified above, the NEI has announced recently in the NIH Guide for Grants and Contracts (June 9, 1978) the availability of a booklet describing opportunities for training in vision research. This booklet contains information identifying the institutional research training programs supported by the National Eye Institute, the addresses and telephone numbers of the program directors, and the major area of research training emphasis of each institutional research training program. To date, we have received several inquiries for this booklet from new investigators.

In considering the continued need for training programs, one must consider whether research opportunities or positions will be available for newly trained investigators. This, to a considerable extent, is based upon available resources. When comparisons are made in Table 2 between the percent of research training funds and the percent of extramural research funds for the Sensory and Motor Disorders of Vision program, there is concern by NEI staff whether the continued level of investment of scarce research training resources in this area is warranted. This concern is compounded by difficulties being encountered by trainees in vision-related neurobiological research on meeting NRSA payback requirements, and on obtaining academic research or teaching positions following their postdoctoral training experience. Our experience in vision-related neurobiological research training and the predominance of Ph.D's being trained in

Table 2

## VISION RESEARCH TRAINING

(\$ amounts in thousands)

PROGRAM	No. Trainees		Amount	No.	Amount	TOTAL	PERCENT TRAINING BUDGET	PERCENT EXTRAMURAL GRANT BUDGET <sup>2/</sup>
	Pre- Doctoral	Post Doctoral						
Retinal and Choroidal Diseases	10	61	\$1,168	44	\$ 572	\$1,740	37%	39%
Corneal Diseases	7	24	578	6	78	596	13%	15%
Cataract	0	7	125	4	52	177	4%	9%
Glaucoma	0	15	278	3	39	317	7%	13%
Sensory and Motor Disorders of Vision	34	47	1,059	58	754	1,813	39%	24%
TOTALS	51	154	\$3,148	115	\$1,495	\$4,643		

<sup>1/</sup> Based on positions awarded for FY 1978. Includes six Graduate Research Training Grants (TOT) to be phased out by June 1979

<sup>2/</sup> By program, percentage of total extramural research grant (R, P, K) dollars for FY 1978

this general area relates also to the National Academy of Sciences Panel on Clinical Sciences and the National Advisory Eye Council's concern about the decline in M.D's being trained on research training programs. Referring to Table 3 there is a substantial decline in the percentage of M.D's being supported under the NEI's institutional training programs.

Table 3

INSTITUTIONAL TRAINING PROGRAMS

	<u>FISCAL YEAR</u>			
	1974	1975	1976	1977
No. T01 Programs <u>1/</u>	48	30	26	14
No. T32 Programs <u>2/</u>	--	23	31	43
Percent M.D's <u>3/</u>	61%	37%	20%	14%
Percent Ph.D's <u>3/</u>	12%	32%	42%	50%
Percent Predoctoral <u>3/</u>	18%	25%	28%	36%

1/ Graduate Research Training Program (T01).

2/ National Research Service Award (NRSA) Institutional Training Programs (T32)

3/ Percent of total trainees (pre- and postdoctoral) under both T01 and T32 programs.

Table 4 indicates that there has been a less dramatic decrease in the number of M.D's supported under individual fellowships during the same time period.

Table 4

INDIVIDUAL FELLOWSHIPS

	<u>FISCAL YEAR</u> <u>1/</u>			
	1974	1975	1976	1977
Percent M.D's	24%	20%	12%	16%
Percent Ph.D's	70%	76%	83%	80%

1/ Postdoctoral only. Includes F02, F03, F22, and F32 awards.

The National Academy of Sciences Panel on Clinical Sciences is currently reviewing the many complex issues associated with this decline and how it may be addressed in terms of the appropriate number of clinicians and health

professionals in biomedical and behavioral research. Concurrently, the National Advisory Eye Council has noted that the greatest need for new vision research personnel is in clinical research. Some of the problems which could account for the decline in number of clinicians entering research training experiences under NEI-supported research training programs are:

1. Willingness of clinicians to spend the time required in a full-time research training experience to become competent and competitive in a particular research field;
2. The decline in real dollars of resources for biomedical research training;
3. Social and financial pressures to pursue health care opportunities;
4. Perceived difficulties in institutional research environments in terms of faculty position opportunities and competitiveness for the available research resources;
5. NRSA payback provisions;
6. NRSA stipend levels and taxability of stipends; and
7. Time span required before funding decisions can be made on fellowships.

Both the National Advisory Eye Council and the NEI have emphasized the great need for advanced clinical research training in specific areas where clinical problems can be delineated and where attention can be paid to the development and evaluation of new methods of diagnosis and treatment. The NEI believes that it is important to support postdoctoral training of clinicians who are willing to spend the necessary time in either a basic science department or a well-staffed clinical research department in which clinical problems related to vision are investigated in association with basic scientists working on analogous problems. In a more modest way, new investigator award mechanisms [Special Visual Sciences Research Awards (R23), Academic Investigator Award (K07)] are attempting to address this problem.

Many of the research training needs identified by the National Advisory Eye Council in its report Vision Research-A National Plan: 1978-1982, are currently being addressed by the NEI. This includes the need for excellence in training and research training environments and the need to encourage health professionals to remain active in vision training research. With the general stability exhibited in research training support under the National Research Service Awards, NEI research training funds will remain relatively constant for the near future, as has been the case for the past five years. To increase the numbers of new individuals entering vision research, the NEI will attempt to complement those individuals being trained through NEI training programs by employing other new investigator support mechanisms to encourage investigators from other disciplines to take advantage of the many research opportunities available in vision research as identified in the report Vision Research-A National Plan: 1978-1982.



## CLINICAL TRIALS

One of the major concerns of those responsible for the conduct of biomedical research in the United States is how to overcome the translation gap which exists between laboratory discovery and a reasonably rapid application of that knowledge to the improved prevention, diagnosis, and treatment of disease. One way of bridging that gap is through the methodology of the clinical trial. Controlled clinical trials are a critical link between biomedical science and health care. These are instances of applied research conducted fully within the milieu of patient care that are unsurpassed as means for validating the safety and efficacy of therapies. The results of clinical trials are meaningful not only to laboratory and clinical investigators but also to health care providers, patients, health educators, officials of regulatory agencies, manufacturers of drugs and devices, administrators of health insurance programs, and others who are concerned with the quality of the health services.

High quality ocular clinical research embodies the same principles and approaches as other areas of basic and applied science. Clinical investigators first identify a problem of interest and formulate hypotheses relative to the potential resolution of the problem. Then they design and conduct investigations to provide definitive tests for the stated hypothesis. Finally, they evaluate their findings in relation to the original hypothesis and its alternatives. Clinical trials, however, differ from laboratory research in that the clinical trial usually deals with large numbers of patients, is very expensive, utilizes extensive resources of manpower, equipment and supplies, takes several years to complete, and often requires the collaboration of several medical facilities throughout the country, working together with a complex administrative framework. Nevertheless, this methodology is invaluable for translating a working hypothesis from the laboratory experiment stage to the clinical research setting, as well as providing a basis for bringing science into the art and practice of medicine.

The National Eye Institute and the National Advisory Eye Council recognize the need for providing resources to the vision research community for the conduct of clinical trials. As reported in last year's Annual Report, most clinical studies which the NEI has supported to date have required the cooperation of several clinical centers in order to acquire the patient population large enough to allow meaningful statistical analysis of the results. These cooperative clinical trials have been supported by research contracts. These major cooperative clinical studies include the trial of photocoagulation for the treatment of proliferative diabetic retinopathy (Diabetic Retinopathy Study), the trial of early versus deferred vitrectomy in diabetic patients with advanced stages of retinopathy (Diabetic Retinopathy Vitrectomy Study), and comparison of the effects of aspirin and photocoagulation in the treatment of early proliferative diabetic retinopathy (Early Treatment of Diabetic Retinopathy Study). An excellent example of the success of such a cooperative clinical trial has recently been reported (Photocoagulation treatment of proliferative diabetic retinopathy: The second report of Diabetic Retinopathy Study findings, *Ophthalmology*, 85:82, 1978).

The NEI considers the research contract the best way of supporting clinical

research when all of the following apply: (1) the topic is one of considerable national importance, (2) NEI staff members and their advisors support a role in developing a concept of the design of the study, (3) the performance of the study requires the collaboration of a number of institutions or clinics, and (4) the NEI has the staff resources to assume responsibility for the management as well as the sponsorship of the study. The NEI has also been receptive to project grant applications for clinical research and will continue to be so.

In the past two years, in response to the high level of interest in clinical trials within the vision research community, the Institute augmented its procedures for reviewing and awarding individual clinical research project grants. Now, whenever any NEI advisors and staff identify a grant application for a proposed clinical trial containing a satisfactory outline of the objectives of the study, addressing the clinical significance of the problem, and demonstrating scientific background and the technical feasibility of the proposed approach, the Institute considers awarding a grant especially for developing and testing a Manual of Operations that accounts for the procedures and conduct of the trial. Once a Manual of Operations has been completed and judged acceptable by NEI staff and advisors, the funds required for execution of the study can be determined and made available via the original grant in accordance with normal NIH procedures. When preliminary work demonstrates that a multiclinic study is required, the original applicants and the potential collaborators at other institutions are encouraged to submit a coordinated set of new grant applications requesting and justifying funds to carry out the study. Because multiclinic trials are significantly more complex and costly than single clinic trials, such cooperative studies are reviewed again through the NIH peer review system. This assists staff and the National Advisory Eye Council in determining whether or not to fund the study.

The NEI-supported clinical trials which have recently successfully completed the Manual of Operations development phase of the study are listed in the table on the following page. In addition to these studies, a number of other clinical trials have been identified by both the National Advisory Eye Council and the NEI staff as being important to vision research and health care. Nine studies are currently in the Manual of Operations development phase. Prior to commencing the clinical trial phase of these studies, the Manuals of Operations being developed for these studies will be reviewed by expert consultants and the Institute staff. Approval for continuing these trials will also be contingent upon the availability of funds.

Whether the clinical trial is supported by grant or contract, the NEI emphasizes the importance of carefully defining certain aspects of clinical research projects before patients are subjected to experimental interventions and observations. Paramount among these are: (1) design of the study and the method for assigning patients to each of various experimental conditions, (2) procedures for reducing investigative bias (masking techniques), (3) criteria for including or excluding patients and for criteria for terminating the study, and (4) statistical techniques that will be used in evaluating the results of the study. In addition, where alternative treatments are to be compared, investigators are to restrict themselves to those instances where, based on knowledge currently available, the risks are comparable, and the estimated benefits of the new intervention should exceed those of the standard therapy. By adopting this approach

and then randomly assigning participants to each experimental group, the investigators not only achieve, with this type of experimental design, scientific rigor, but also attain a high ethical standard because every participant has an equal chance of receiving whichever of the therapies is eventually found to be best.

To assist in the monitoring of the grant-supported clinical trials and the identification of new clinical research findings which may be transferred to the benefit of the public, the NEI plans, in the spring of 1979, to conduct the first in a series of annual meetings of grant-supported clinical trials. This meeting will involve those individuals who are currently in the clinical trial phase or the Manual of Operations Development phase of their NEI-supported clinical trials. The purpose of this meeting will be to discuss the various study designs of the projects, the problems encountered in the implementation phase of the studies, the solutions to these problems, and the current status of the projects to date. A report of this meeting will be made available to the public.

Such a meeting will assist in fostering the evaluation of new and established medical and surgical interventions, in encouraging the coordinated dissemination of clinical research results, and to insure that this data is appropriately processed for effective transfer to the eye and vision care community. It should be noted, however, that this expansion of clinical trials and knowledge transfer activities will not be done at the expense of the continued growth and development of NEI's support of basic and applied research. Otherwise, there will be no new knowledge to invest or transfer.

NEI GRANT SUPPORTED CLINICAL TRIALS

CLINICAL TRIAL PHASE

TRIAL	INSTITUTION	INVESTIGATOR
Effect of Vitamin E on Prevention/Reduction of Retrolental Fibroplasia in Premature Infants	Pennsylvania Hospital	Thomas R. Boggs, M.D.
Effect of Photocoagulation in Shortening the Duration of Central Serous Chorioretinopathy	Mayo Clinic	Dennis M. Robertson, M.D.
Effect of Photocoagulation in the Treatment of Senile Macular Degeneration	Moorfields (England)	Alan C. Bird, M.D., F.R.C.S.
Effect of Photocoagulation on the Treatment of Branch Vein Occlusion <u>1/</u>	Johns Hopkins University University of Illinois University of Miami Eye Research Institute University of Southern California	Daniel Finkelstein, M.D. David H. Orth, M.D. John Clarkson, M.D. Clement L. Trempe, M.D. Arthur W. Allen, M.D.
Use of Orthokeratology in Changing Corneal Curvature to Reduce Refractive Error	University of California, Berkeley	Kenneth A. Polse, O.D., M.S.

1/ Multicenter grant supported clinical trial (R10).

## VISION RESEARCH CENTERS

The historical background of National Eye Institute support of vision research centers as well as the National Eye Institute center concept was discussed in detail in last year's Annual Report. The NEI currently employs only two types of large center grants, the Specialized Clinical Research Center Grant (P50), and the Core Grant for Vision Research Centers (P30). Table 1 provides a summary of NEI vision research centers for FY 1978.

Table 1

<u>Center</u>	<u>Number</u>	<u>Amount</u>	<u>% ECP</u> <u>1/</u>
P 30	19 <u>2/</u>	\$ 3,003,005	4%
P 50	7 <u>3/</u>	1,523,325	2%

Key to abbreviations: P30 = Core Grants for Vision Research Centers  
P50 = Specialized Clinical Research Center Grants  
ECP = Extramural and Collaborative Programs

- 1/ FY 1978 ECP budget (grants, centers, academic investigator and research career development awards, contracts, research training) = \$74,373,000.
- 2/ Includes four new P30's: Bascom Palmer Eye Institute, University of Illinois, University of California, and Massachusetts Institute of Technology.
- 3/ Includes two new P50's: New York University Medical Center and Massachusetts Eye and Ear Infirmary.

Only 6% of the NEI's Fiscal Year 1978 Extramural and Collaborative Program's research budget went for support of the center grants.

As indicated in Table 2, the NEI currently supports seven specialized clinical research centers:

Table 2

### SPECIALIZED CLINICAL RESEARCH CENTERS

<u>NEI Program</u>	<u>Institution</u>	<u>Clinical Research Emphasis</u>
<u>Retinal and Choroidal Diseases</u>	Bascom Palmer Eye Institute, University of Miami	Macular Disease
	Massachusetts Eye and Ear Infirmary	Retinitis Pigmentosa

<u>NEI Program</u>	<u>Institution</u>	<u>Clinical Research Emphasis</u>
	New York University Medical Center	Hereditary Retinal Degenerations
<u>Corneal Diseases</u>	University of Florida	External Ocular Diseases
	Eye and Ear Hospital of Pittsburgh	Ocular Autoimmune Phenomenon
<u>Glaucoma</u>	Washington University	Glaucoma
	Massachusetts Eye and Ear Infirmary	Secondary Glaucomas

These centers have several attributes. Investigators can conduct innovative scientific studies in a health care setting and in an environment which allows the full promise of these investigator's research capabilities to come to fruition. Specifically, clinical centers feature outstanding clinical expertise, facilities, and excellent referral arrangements that assure a steady flow of patients with disorders that require study centers. These investigators are committed to bring the latest laboratory methods to the clinical research setting, and they have well-developed insights about the theory and practice of biostatistical and epidemiologic techniques.

The NEI also supports core grants at 19 institutions heavily engaged in vision research. The following table summarizes the core grants currently supported by the NEI, the number of NEI grantees sharing the core resource facilities, and an indication of the interdepartmental cooperation and collaboration facilitated by the core center facilities.

Table 3

CORE GRANTS FOR VISION RESEARCH CENTERS (P30)

<u>Institution</u>	<u>Number of NEI Grantees Using Core Facilities</u>	<u>Examples of Interdepartmental collaboration</u>
University of Washington Department of Ophthalmology	17	Biological Department, Physiology, Anesthesiology, Psychiatry, Psychology, Obstetrics and Gynecology
Johns Hopkins Hospital Wilmer Ophthalmological Institute	18	
University of Pennsylvania Institute of Neurological Sciences	9	Anatomy, Veterinary Medicine

<u>Institution</u>	<u>Number of NEI Grantees Using Core Facilities</u>	<u>Examples of Interdepart- mental Collaboration</u>
New York University Department of Ophthalmology	9	Pathology, Immunology
Massachusetts Eye and Ear Infirmary Howe Laboratory of Ophthalmology	8	Physiology, Anatomy, Pathol- ogy, Neurology, Pharma- cology
University of Miami Department of Ophthalmology	9	Epidemiology, Biostatistics, Biophysics Laboratory
University of Illinois Department of Ophthalmology	11	Dermatology, Pathology, Mole- cular Biophysics, Pharma- cology
University of California, San Francisco Francis I. Proctor Foundation for Research in Ophthalmology	28	
Medical College of Wisconsin Ophthalmology and Physiology Departments	3	Pediatrics, Wood Veterans Administration Center
Columbia University Department of Ophthalmology	15	
Mount Sinai School of Medicine Department of Ophthalmology	8	Physiology, Pharmacology
University of California, Los Angeles Jules Stein Eye Institute	16	
Eye Research Institute of Retina Foundation, Boston	25	
Yale University Department of Ophthalmology and Visual Science	11	Physiology, Biophysics, Pharmacology
University of California, San Francisco	28	Pathology, Biochemistry, Physiology, Pharmacology
Institutes of Medical Sciences Smith-Kettlewell Institute	6	Neurophysiology, Pharmacology

<u>Institution</u>	<u>Number of NEI Grantees Using Core Facilities</u>	<u>Examples of Interdepart- mental collaboration</u>
Harvard Medical School	19	Physiology, Anatomy
University of Rochester Center for Visual Sciences	8	Anatomy, Psychology
Massachusetts Institute of Technology Department of Psychology	8	New College of Health Sciences and Technology; Clinical Medicine, Beth Israel Hospital; Children's Hospital; Massachusetts Eye and Ear Infirmary; Tufts; Eye Research Institute of Retina Foundation; New England College of Optometry.

By providing support for an essential nucleus or centralized core of resources facilities and services which are shared by investigators in a number of individual research projects, this mechanism provides an opportunity to achieve four key objectives: (1) to afford researchers greater opportunity and flexibility by integrating commonly required research resources, (2) to enhance research capabilities and productivity by strengthening the research environment, (3) to facilitate multidisciplinary approaches to specific problems in the visual sciences, and (4) to promote interaction and collaboration between vision scientists and researchers in areas outside the visual sciences.

The NEI, with the assistance of the National Advisory Eye Council, has recently revised its Core Grant guidelines, in order to define policy requirements clearly. The major policy issues stated in the guidelines include the following: (1) The institution must be engaged in a substantial volume of research in the visual sciences. This means that at least four investigators must have NEI project support and that there be no less than six NEI supported research grants. This would be the initial threshold of eligibility for a core research center. (2) The proposed center research team must demonstrate that interdisciplinary collaborative efforts, both laboratory and clinical, among investigators and departments will achieve research advances not possible or justifiable through the activities of the individual project grants alone. (3) The core center activities will facilitate a multidisciplinary research approach to specific problems in the visual sciences. (4) There must be an institutional commitment to the core research center enterprise which is being proposed. (5) The core research center proposal must be in conformance with Public Health Service policy grant statements. We are stressing in these revised guidelines that core grants do not provide direct funding or supplementary support for individual research projects. Also, once the threshold for core grant eligibility has been met, in terms of the number of active NEI research project grants, the level of core support is based primarily upon the prospect of achieving the objectives identified previously rather than of a formula related to the total number, or dollar level of active NEI grants at an institution.

Noting that the Council's report Vision Research--A National Plan: 1978-1982 calls for continued efforts to promote high quality clinical research with

special emphasis on the regular research project grant, the present type of clinical center grant (P50) employed by the NEI may not achieve all the objectives identified by the NEI and the National Advisory Eye Council because of the following reasons: (1) the full range of clinical approaches is not occurring under this center mechanism, (2) several of the studies being supported are descriptive or correlational efforts with small sample sizes, and (3) there are very few well-designed natural history studies. Another reason for concern is that applicants, reviewers, and NEI staff have had difficulty in determining how much documentation should be required for such centers in terms of details for the research plans. As a result, there has been some discussion by the National Advisory Eye Council and NEI staff regarding the development of a Specialized Clinical Research Center Development Award. The purpose of such a development award would be to strengthen the clinical research capabilities of eye care centers and other centers for vision sciences. In general, funds would be provided to enable clinical investigators to: (1) develop the complement of staff, facilities, and collaborative relationships which are required to plan and conduct high quality clinical research relevant to abnormalities of the visual system, (2) to transform protocols for specific clinical research projects into detailed experimental designs and manuals of operation, and (3) to demonstrate the feasibility of studies to be described in the manual of operations. The details of this development award are currently being developed by NEI staff with guidance from the National Advisory Eye Council. Additional details regarding this development award and the plans for its implementation will be made available in next year's Annual Report.

Because its primary mechanism for support of vision research is the individual project grant, the NEI uses center grants judiciously. An operational range for support of vision research centers (P50, P30) is between 6% and 8% of the total extramural budget of the NEI. This will maintain sufficient program budget flexibility to take advantage via the investigator-initiated grant of the many research opportunities identified by the National Advisory Eye Council in its report Vision Research--A National Plan: 1978-1982.



### Introduction

Research in retinal and choroidal diseases involves broad areas of investigation which concern development, structure, function, and degeneration. Knowledge in these areas is essential to improved understanding of the etiology, diagnosis, and treatment of clinical disorders.

Many causes of blindness relate to the loss of retinal function. The outer segments of the rods and cones are structurally and functionally dependent upon the retinal pigment epithelium. If the photoreceptors become detached from the pigment epithelium through trauma or cellular dysfunction, a clinical entity known as retinal detachment will occur with an ensuing loss of sight. Disorders of the visual cells and pigment epithelium include a multiplicity of developmental and degenerative disorders which involve specific structural components and chemical processes. These disorders include retinitis pigmentosa, macular degeneration, and retinal detachment. Research in this area is directed toward better understanding of the fundamental processes and interactions of the photoreceptors and pigment epithelium. New knowledge will be applied to the early diagnosis, prevention, and treatment of retinal disorders.

The recent report of the National Advisory Eye Council, Vision Research-A National Plan: 1978-1982, emphasized the need for more studies of animal models of human retinal diseases as a means of advancing the knowledge which is needed for the management and prevention of retinal diseases.<sup>1</sup> Areas of research emphasized in the plan include: (1) Pigment epithelium, its development and genetic regulation; its role in nutrition and normal retinal function; and its congenital acquired dysfunctions; (2) Intracellular recording and functional properties of retinal neurons in intact systems; (3) Study of visual pigments in transduction processes; (4) Mechanisms of retinal induction, differentiation, and synaptogenesis; (5) Factors which preserve the adherence of retina to pigment epithelium; and (6) Development and evaluation of natural and synthetic vitreous substitutes.

### Visual Cell Renewal and Autophagy

The human diseases which are classified as retinitis pigmentosa are characterized by a loss of vision due to degeneration of photoreceptors and an invasion of the neural retina by the pigment epithelium. Because photoreceptor dysfunction is a major cause of blindness, it is important to understand the metabolism of these photoreceptors and the pathogenic process. The retinal pigment epithelium has numerous roles which are necessary for the maintenance of the normal functions of retinal photoreceptors. For instance, the retinal pigment epithelium is involved in the transport of ions and nutrients, in vitamin A metabolism, and in the photoreceptor renewal process. Among the objectives of NEI's Retinal and Choroidal Diseases program is learning more about the biochemical, physiological, and structural interactions of visual

cells and retinal pigment epithelium.<sup>1</sup>

### Cone Shedding

In the mammalian retina there is an intimate relationship between the photoreceptors and the pigment epithelium which has been described by transmission and scanning electron microscopy. For the most part there is more information about the rod-pigment epithelium relationship than about the cone-pigment epithelium. The association of the cones with the pigment epithelium and phagocytosis of cones in human retina has been studied by Steinberg and associates.<sup>2-5</sup> These investigators have found villous-like apical processes protruding from the pigment epithelium into space above each cone. Some of the processes are in a sheet-like form which ensheaths the upper one-third of the outer segment. The human cone sheath consists of three or four overlapping sheaths and may function to isolate cones from rods. There are organelles which include mitochondria in the sheaths, and their presence suggests an active metabolic exchange between the sheath and photoreceptor. The presence of the sheath suggests that it may be involved in the phagocytosis of shed packets of discs from tips of cones in a manner similar to the process described in rods.

### Cyclic Nature of Cone Shedding

Young and associates<sup>6-8</sup> reasoned that cones might shed their outer segments after they have undergone a long period of daylight and after the onset of darkness. Their reasoning was based on the observations of the cyclic nature of rod shedding, as first reported by LaVail and associates.<sup>9-11</sup> The time of shedding of cones explains why the phenomenon had not been observed before. These new observations have been reviewed recently.<sup>12</sup>

The control mechanisms involved in the rhythmic shedding of photoreceptor discs remain to be elucidated. The existence of daily rhythms in visual cells may have importance in the pharmacological treatment of clinical disorders. These findings open new fields of investigation into the effect of light in disorders such as macular degeneration and retinitis pigmentosa.<sup>1</sup>

### Cone Outer Segment Renewal

The outer segments of cones and rods have different anatomical features. Discs in cones are open to extracellular space while only the basal discs in rods are open. Autoradiographic studies show differences in renewal of outer segments. While rods show an outward displacement of newly assembled discs, cones appear to be renewed by replacement of molecules in existing membranes. The concept of molecular renewal of cones is supported by the absence of phagosomes which are derived from cone outer segments and identifiable within pigment epithelium. Studies of cone renewal and shedding have been conducted in laboratory animals kept under daylight conditions.

Observations of cone disc shedding in humans and of the rhythmic nature of disc shedding support the current view that both rods and cones renew their outer segments by a process of membrane replacement. The absence of "moving-bands" in autoradiographic studies of protein renewal in cones is now explained on the basis of structural rather than metabolic differences between rods and cones. Labelled proteins can diffuse within the entire cone outer segment because cone membranes are continuous and not individual discs, as are those of rods. Species investigated include squirrel, cat, rhesus monkey, and man; all show evidence of cone disc shedding and new membrane assembly similar to that which occurs in rods. The cone system is not as well understood as the rod system,<sup>1</sup> and among the new issues are whether the same factors governing assembly and shedding of discs are involved in both rods and cones.<sup>8</sup> An interesting suggestion made by Steinberg and associates<sup>5</sup> is that concerns about toxic effects of light on the human retina may be exaggerated to the extent that the shedding of cone discs and retinal pigment epithelium phagocytosis are normal ongoing processes and not conclusively an indication of degenerative changes. Fisher and associates<sup>13</sup> are continuing their observations of developing human cones because of the ability of mammalian adult cones to synthesize new discs. The project is designed to damage cone outer segments and to study their regenerative capacities in the ground squirrel as the animal model. A xenon arc source is used to produce focal damage in one group of animals, and vitamin A deficiency is used to produce cone degeneration in another group. This study is in progress; however, preliminary studies have been done on rhesus monkey and cat. Disc shedding was found in cones of these animals. Structural relationships between cone outer segments and pigment epithelium show that phagosomes from cones are transported through long apical pigment epithelial processes which contact cones. These structural models for mammalian cone outer segment shedding will receive further attention because of differences in the rate of degeneration of cone and rods in retinitis pigmentosa.<sup>1</sup>

### Vitreous Humor and Vitreoretinal Disorders

Vitreous degeneration and retinal detachment are disorders which involve the management of rhegmatogenous retinal detachment, removal of opaque vitreous, diffusion of substances, and adherence of vitreous to neural retina.

A leading cause of new blindness in this country today is diabetes mellitus which involves deterioration of the blood vessels, an event which is particularly dangerous for the eye.<sup>1</sup> In virtually every case of blindness in the diabetic, the vitreous humor may play a direct and causative role. This is mainly due to its limited ability to clear an intraocular hemorrhage. When a retinal blood vessel breaks and blood flows into the vitreous humor, vision is immediately impaired: light cannot get through the vitreous to the retina. Blood in the vitreous acts as an irritant.

Through a series of inflammatory reactions, scars may develop and permanently impair vision. Through contraction of the vitreous,

the retina may pull away from its normal position and cause retinal detachment. A similar series of events can follow the vascular complications of retrolental fibroplasia of infants, traumatic vitreous hemorrhage, and sickle cell retinopathy. The detachment can cause permanent impairment of vision. These factors may cause a retinal detachment: fluid accumulation under the retina; abnormal traction exerted on the retina in the direction of the vitreous cavity; and tears or holes in the continuity of the retina which permit fluid vitreous to reach the subretinal space and to float the retina off the pigment epithelium. Clinically, the most frequent type of detachment is rhegmatogenous, which is one where retinal breaks coexist with a variable amount of vitreous traction.<sup>1</sup>

The introduction of vitrectomy as a means of treating blinding diseases has allowed the procurement and detailed analyses of vitreous specimens.<sup>14</sup> The human vitreous is normally a gel structure which becomes liquefied with age, following trauma, and in many disease states. Little is known about vitreous as found in diabetes, massive periretinal proliferation, macular puckers, and vitreous retraction phenomenon. Although it is recognized that structural proteins of the vitreous are responsible for the gel state, little is understood about the structure, biosynthesis, and interactions of the vitreous structural constituents.

Balazs and associates<sup>15</sup> have been conducting a multidisciplinary approach to the study of causes of vitreoretinal disorders. This investigation is an effort to understand the structure and function of vitreous as well as fundamental work on the biological function of hyaluronic acid in relation to vitreous. The project includes preclinical testing of the therapeutic effect of hyaluronic acid preparations. Possible clinical application of hyaluronic acid includes replacement of pathological vitreous and use as an anti-inflammatory agent to prevent fibrous tissue and adhesions.

It has been noted in a recent review that a number of substances such as gases, salt solutions, oils, synthetic polymers, and some body fluids have been used to replace vitreous after intraocular surgery.<sup>16</sup> However, the search for the ideal vitreous replacement substance continues.<sup>1</sup> In general, substances to be used for restitution of vitreous space should have physical properties similar to those of vitreous and be physiologically compatible with vitreous and other eye tissues.

### Studies on Vitreous Cells

Hyalocytes are cells which are embedded within the vitreous gel. A detailed comprehension of their metabolic regulation and role may give insight into the production of abnormal vitreous. Changes in the anabolism or catabolism of the hyaluronic acid or collagen of vitreous may lead to alterations in rheological and optical properties of vitreous.

As a first step in the study of the regulation of hyaluronic acid synthesis by hyalocytes, Jacobson<sup>17</sup> and Hultsch<sup>18</sup> and associates have

initiated efforts to establish optimal conditions for the isolation and culture of cells from bovine and rabbit vitreous. Hyalocytes will be: (1) isolated from vitreous by combined collagenase/hyaluronidase digestion; (2) sedimented, washed, and suspended in culture medium for in vitro studies; and (3) cultivated in the presence of radioactive precursors to determine the rate of formation of extracellular matrices. Such experiments may provide essential basic information about the possible role of extracellular substances such as steroid hormones, ascorbic acid, and purified hyaluronic acid on the synthetic properties of cultured hyalocytes.

### Effects of Aging

Balazs and associates<sup>15</sup> have analyzed vitreous from nearly 1,000 eyes from humans between the ages of 20 to 90 years. They find that the aging process is different from that assumed in the research literature in that liquid vitreous is found in the young adult as well as in elderly humans. However, the distribution of liquid vitreous changes during the aging process. The vitreous of rhesus monkey is closest in physical and chemical structure to that of man. Therefore, a sampling from a large number of rhesus monkeys and slit lamp examinations are planned in order to obtain sufficient material for study.

Jacobson and associates<sup>17</sup> have applied the techniques of in vitro studies of hyalocytes to observations of effects of aging on the biosynthesis of vitreous hyaluronic acid. Previous studies have utilized enzymes isolated from hyalocytes obtained from calves of approximately four months of age. Because there is a dearth of information concerning the metabolism of vitreous hyaluronic acid as a function of aging, these investigators have isolated hyalocytes from the vitreous of cows two to four years of age. The results are preliminary but suggest that the enzyme activities are low in cow hyalocytes. The results also indicate that the function of soluble, hyaluronic acid-associated enzymes may be to resynthesize and repair lost or denatured vitreous and that low levels of new hyaluronic acid are synthesized in the normal vitreous of the mature cow. Perhaps synthesis of new vitreous in bovine is age-related and limited to embryonic and postnatal development, whereas, in the owl monkey, hyaluronic acid in vitreous is known to regenerate fully and is subject to regulatory feedback mechanisms.

### Vitreous Collagen

Approximately 85 percent of the insoluble structural proteins in bovine vitreous is identifiable as collagen. It is recognized that collagen has a role in the maintenance of the gel state of vitreous and that the collagen network is stabilized by hyaluronic acid. The ability of vitreous to play a mechanical role in the determination of eye shape and size is demonstrated by removal or dissociation of collagen, resulting in liquefaction of the vitreous gel, and is related to the unique fibrous form of the vitreous collagen fiber and structures.

Swann and associates<sup>19-21</sup> have solubilized vitreous collagen by

treatment with proteolytic enzymes and have shown that this collagen has an amino acid composition similar to collagen present in cartilage, although there are detectable differences between helical structures in vitreous and cartilage collagen. These studies should provide valuable information about the basic mechanisms which regulate the aggregation of collagen molecules to form fibers in vitreous and the role of vitreous in the management of retinal disease.<sup>1</sup>

#### Transport of Vitamin A

The pathological similarities observed in photoreceptors from animals with hereditary degenerative disorders and animals maintained on vitamin A deficient diets have stimulated research on the function of vitamin A and its transport to functional sites. Nearly all steps involved in the absorption of vitamin A and its precursors and their transport to the retina have been examined in retinitis pigmentosa patients. There is no evidence that the vitamin A transport process is affected in these patients. Nevertheless, as more details of the transport process are described and as more components of the complex vitamin A uptake are understood, the possibility becomes greater that vitamin A transport defects in animal models and in patients may be found. The prime targets of destructive disease processes may be at the level of membranes of photoreceptors and retinal pigment epithelium.<sup>1</sup>

Several laboratories are investigating the biochemical role of intracellular binding proteins for the vitamin A derivatives retinol, retinal, and retinoic acid in retina and retinal pigment epithelium.<sup>22-24</sup> Vitamin A is insoluble and unstable in tissues; therefore, binding proteins serve to protect vitamin A and ensure its delivery and concentration at functional sites.

Saari and Futterman and associates<sup>25</sup> have shown that extracts of bovine retinal pigment epithelium and neural retina contain large amounts of cellular retinol-binding protein and no detectable cellular retinoic acid-binding protein. The retinol-binding proteins of both tissues are similar with respect to molecular weights and binding specificity. The photoreceptor outer segments contain approximately 10 percent of the retinol-binding protein found in the neural retina. The location of the major portion of retinol-binding protein has not been conclusively shown, but speculation is that it may be distributed in other portions of the photoreceptors where the derivative of vitamin A is utilized in the biosynthesis of rhodopsin.

Heller and associates<sup>26</sup> have used isotope labelled retinol-binding protein to demonstrate that in the transport process the retinal pigment epithelium cells show a polarity where the label was confined to the choroidal surface of the cell. The retinol-binding protein receptors are present on the basal and lateral plasma membrane of the retinal pigment epithelial cells. The transport of retinol and retinoic acid is rapid and is a temperature-dependent process. The transport of vitamin A is more complex and subject to controls not previously appreciated. Among

the issues raised by these studies is that in retinitis pigmentosa the defect causing impaired vitamin A transport might be at the receptor sites. It seems clear that further investigation of the dynamic properties of membranes is needed.<sup>1</sup>

### Hereditary Retinal Degeneration

Approximately one-third of all blindness among school age children in the United States can be attributed to hereditary or developmental eye disorders. Retinitis pigmentosa accounts for a major proportion. Its manifestations, including progressive night blindness and loss of visual field, are correlated with the nature of the genetic defect. In addition, distinct effects observable in the electroretinogram have also been correlated with the nature of the genetic defect.<sup>27</sup>

At the NEI-supported Specialized Clinical Research Center for the Study of Retinitis Pigmentosa and Allied Diseases at the Massachusetts Eye and Ear Infirmary, Harvard Medical School, an interdisciplinary team of an ophthalmologist, anatomist, biochemist, cell biologist, physiologist, and biostatistician are working toward improved understanding of these relationships. They are studying the natural histories of these disorders in a large cohort of patients using modern techniques for measurement of visual function<sup>28</sup> and for genetic analysis. In addition, through an eye-donor program, relationships between the clinical manifestations and the pathology in the eye are being examined via electronmicroscopic,<sup>29</sup> biochemical,<sup>30</sup> and tissue culture studies.

During this past year, the National Eye Institute funded a Clinical Research Center for Heredo-Retinal Degenerations at the New York University Medical Center. This center is dedicated to quantitative evaluation of the developmental course of hereditary retinal diseases and identification of the initial pathological abnormalities. A battery of noninvasive tests of retinal and pigment epithelial function is employed. Included are the latest sophisticated techniques for fundus reflectometry, focal electroretinography, visually evoked cortical responses, psychophysical studies of retinal receptive field interactions, and spatial contrast sensitivity thresholds.

The importance of fundamental studies to the improved understanding and management of clinical entities has been demonstrated by recent advances in knowledge with respect to gyrate atrophy, a degenerative disorder of the choroid and retina. Following the initial finding of a biochemical defect (an elevated level of ornithine) in patients with gyrate atrophy,<sup>31</sup> a number of investigators have gone on to demonstrate that a single enzyme, ornithine ketoacid transaminase, may be at fault.<sup>32,33,34</sup> Investigators are now developing plans for clinical studies of therapeutic interventions based on these findings.

In the absence of clinical pathology material from patients, knowledge of the actual degenerative process in the retina can be obtained only through noninvasive procedures. Even when pathologic

samples are available, however, they usually demonstrate the end results of the degenerative process. In order to "observe" with morphologic, biochemical, and biophysical techniques the progress of degenerative processes in the retina during development, and in order to make comparisons with normal developmental processes, animal models must be employed. Two major programs of animal resource development for the study of hereditary retinal degenerations are being supported by the National Eye Institute, one at the University of Pennsylvania and one at the University of California at San Francisco. Both programs have formed active collaborations with a number of NEI-supported investigators across the United States who are studying normal retinal development and the retinal-degenerative processes related to various diseases. At the University of California, LaVail and his associates are continuing their studies on various congenic strains of rats derived from the RCS dystrophic rat. As described above major emphasis is given to the interaction between photoreceptor cells and pigment epithelium cells as this is likely the primary aspect of the defect in retinitis pigmentosa and other retinal degenerative disorders. The studies at the University of California on normal and dystrophic rats include evaluation of the effects of lighting, environmental, and genetic factors.<sup>35</sup>

Studies with a number of breeds of dog are being conducted at the University of Pennsylvania.<sup>36</sup> Aguirre and his associates have identified hereditary photoreceptor defects in the miniature poodle, Irish setter, Norwegian elkhound, and other dogs. The manifestations of the hereditary disturbance varies between the various species of dogs; some primarily exhibit rod involvement, others primarily cone involvement, and others both rod and cone involvement.

The primary research objective, using these dogs as models, is to characterize further the nature of the defect in each case. For example, in a collaborative study between investigators at the University of California at Los Angeles and at the National Eye Institute, it was recently demonstrated that the affected visual cells of Irish setter dogs show: (1) a deficiency in cyclic GMP phosphodiesterase activity and (2) an elevated level of cyclic GMP.<sup>37</sup> Defects in cyclic GMP metabolism have been identified in other models of retinal degeneration<sup>38</sup> and may, in particular, be implicated in the retinal degenerations that cause blindness early in life.

The National Eye Institute recognizes the importance of animal models to research on developmental and hereditary disorders of the eye. Some of the progress in recent years in research directed at understanding the nature of the degenerative process (as well as normal processes) can be attributed to NEI's efforts to make RCS rats available to a number of investigators. This has been made possible through a contract program for the development, production, and distribution of these animals. An additional initiative during the past year has been the commitment of the National Eye Institute to a similar program under which strains of dogs with retinal degenerative disorders similar to those of man are being developed and made available to qualified investigators on a nationwide basis.

## Diabetic Retinopathy and Other Vascular Disorders of the Retina

Vascular and circulatory abnormalities are the leading causes of new blindness and visual impairment in the United States today. The most prevalent of such disorders is diabetic retinopathy; others are retinopathy of prematurity (retrolental fibroplasia), and the vascular occlusions (central vein occlusion, branch vein occlusion, and retinal artery occlusion). In the past decade, there have been two major advances, one in diagnosis and one in treatment, which have had major impact on research directed at better understanding of the etiology and pathophysiology of retinal vascular disorders and on research directed at improved clinical management of these disorders: (1) Fluorescein angiography has provided an effective and safe technique for evaluating the status of the retinal circulation through visualization of sites of retinal blood vessel closure, the extent and severity of retinal blood vessel leakage, and the extent and severity of new blood vessel growth. Investigators at a number of leading eye research centers are currently actively seeking improved materials and techniques which will carry angiography beyond its current resolving capability.<sup>39,40,41</sup> In addition, new techniques such as laser velocimetry<sup>42</sup> are also being evaluated for their potential in improving diagnosis. (2) Photocoagulation therapy (laser and xenon arc) has been developed and utilized as the principal technique for treating disorders of the retinal blood vessels.<sup>1</sup>

Because of the magnitude of the public health problems posed by retinal vascular disorders, and the research community's recognition of the need and opportunity for their solution, approximately 14% of the NEI's FY1978 budget went to support research on these disorders. Most of this support went for nearly 100 investigator-initiated research grants for fundamental and clinical investigations.

A number of vision scientists have been examining the hypothesis that the abnormal proliferation of retinal vessels commonly observed in the proliferative retinopathies (such as diabetic retinopathy and retrolental fibroplasia) is due to the production of a vasoproliferative substance that stimulates an ingrowth of vessels. Suggestive evidence comes from a number of recent findings. For example, investigators at Johns Hopkins University have shown that while the total soluble vitreous protein in puppies rapidly decreases as the eye matures, exposure to oxygen at levels that produce retinal capillary closure results in a significant retention of these proteins.<sup>43</sup> Further biochemical studies are being conducted in order to determine whether or not these effects are related to vasoproliferative factor.

Other studies have concentrated on substances that might inhibit neovascularization. From studies on the induction of neovascularization with tumor aminogenesis factor (TAF) it has been found that neovascularization occurs when tumor cells are in direct contact with the retina but not when they are as little as 100 microns from the retina.<sup>44</sup> This finding is unique to the retina as TAF usually stimulates new vessel formation at distances of several millimeters. This suggests the

possibility that there might be an inhibitor of neovascularization present in the vitreous. The nature of this putative substance and its role in the normal and disease states require further investigation.

Other studies are concentrating on the fine structure of the retinal vessels and associated structures with the aim of identifying the structural and biochemical bases for retinal vascular disorders. For example, a common finding in diabetic blood vessels is that the basal laminae become thickened. Does this represent increased synthesis of material, a failure of normal degradative processes, or the presence of abnormal chemical substances for which normal turnover mechanisms are inoperative? A major problem in dealing with this question relates to the need for techniques which will make possible the analysis of small amounts of tissue from individual human retinæ. Investigators at the University of Arizona have recently refined techniques which permit the isolation of purified retinal vascular membrane from small amounts of tissue which can be used for both morphological and chemical investigation.<sup>45</sup> These investigators are now embarking on a comparison of extracts of basement membrane from normal and diabetic human eyes obtained through an eye bank. Biochemical findings will be examined in relationship to histological, morphological, and basement membrane thickness measurements on the retinæ from which the material was isolated.

At the Eye Research Institute of Retina Foundation, Boston, investigators are also looking at the fine chemical structure of diabetic human basement membrane.<sup>46</sup> They have worked out methods for quantitating carbohydrates, glucosylgalactosylhydroxylysine and galactosylhydroxylysine in single human lens capsules. Application of these techniques in the comparison of ocular basement membranes from diabetics with those from normals will likely also lead to improved understanding of the etiopathology of retinal vascular disorders.

Diabetic retinopathy is part of the generalized microangiopathy associated with diabetes in which the small blood vessels lose their normal vascular tone and show increased permeability. With respect to the former, Cunha-Vaz and his associates<sup>47</sup> present evidence to indicate that decreased vascular resistance in capillaries and venules and increased arteriolar constriction may account for the increased blood flow (decreased mean transit time) seen in diabetic retinopathy. With respect to the latter, investigators at Washington University,<sup>48</sup> using vitreous fluorophotometry, have recently presented additional evidence to demonstrate that the increased permeability of retinal vessels in microangiopathy is associated with defects in the blood-retinal barrier early in human diabetic retinopathy. It appears that these defects may be in the tight membrane junctures which normally do not allow fluorescein to leak through.<sup>49</sup>

The early abnormalities in the structure of the blood-retina barrier in diabetes are poorly understood. Promising results have recently been obtained from studies on dogs with induced diabetes. Investigators at the University of Wisconsin have recently presented data to indicate that it is the opening of endothelial tight junctions which provides

the pathway for leakage.<sup>50</sup> Additional problems now remain to be examined. For example, studies need to be conducted on the time course of the leakage, the sites of leakage within the retinal vascular tree, the pore sizes of leaking channels, the nature of the leaking substance, the effects of leakage on further vascular changes such as basement membrane thickening, and the effects of diabetic control.

During the past year, the National Eye Institute has initiated special program efforts with respect to four types of retinal vascular disorders: diabetic retinopathy, branch vein occlusion, central serous chorioretinopathy, and retinopathy of prematurity. These are discussed in the following section.

Diabetic Retinopathy. In other portions of this Annual Report, the accomplishments in the Diabetic Retinopathy Photocoagulation Study,<sup>51</sup> the status of the Diabetic Retinopathy Vitrectomy Study, and the initiation of the Early Treatment of Diabetic Retinopathy Study are discussed. In addition to these activities, the National Eye Institute has participated in a number of trans-NIH ventures associated with the expanded national program of basic and clinical research into the cause, cure, and prevention of diabetes mellitus and related disorders. Representative of these has been the National Eye Institute's participation, together with seven other NIH Institutes, in making program announcements requesting the submission of research grant applications to study diabetes mellitus and related problems<sup>52</sup> and to study the epidemiology of diabetes.<sup>53</sup>

Branch Vein Occlusion. The Diabetic Retinopathy Study has demonstrated the value of photocoagulation for certain stages of proliferative retinopathy associated with diabetes. Obstruction of a major branch vein may also result in retinal neovascularization and macular edema. While there is considerable suggestive evidence that photocoagulation therapy might be of benefit in reducing the loss of vision due to branch vein occlusion, its efficacy is uncertain; no carefully controlled studies have been performed. Recognizing this need, the National Eye Institute awarded a grant to investigators at Johns Hopkins Hospital in 1977 to develop the protocol to conduct a clinical trial to assess the efficacy of photocoagulation in treatment of branch vein occlusion. In June 1978, the manual of operations was completed,<sup>54</sup> and awards have been made to five clinical centers for participation in a study of the natural history of branch vein occlusion and the efficacy of photocoagulation in its treatment: Wilmer Ophthalmological Institute, Johns Hopkins Hospital; University of Illinois Eye and Ear Infirmary; Bascom Palmer Eye Institute, University of Miami; University of Southern California; and the Eye Research Institute of Retina Foundation, Boston.

Central Serous Chorioretinopathy. The major characteristic of this disorder is an accumulation of serous fluid between the sensory-cell layer of the retina and the retinal pigment epithelium. The defect is thought to be produced by leakage of serum from within the choroid and across Bruch's membrane. The disorder, usually monocular, primarily afflicts men between the ages of 20 to 40 years with symptoms which include loss of central visual function, image distortion, impaired

visual space perception, and disturbances of color vision and dark adaptation.

In November 1977, investigators at the Mayo Clinic initiated a randomized controlled clinical trial to test the efficacy of photo-coagulation therapy in the early resolution of this disorder.<sup>55</sup> The investigators are also evaluating whether photocoagulation can be used to create an outflow path for accumulated serous fluid in addition to (or instead of) its use in sealing "leaks" in the pigment epithelium. Recruitment of the 90 patients required for this single-center study is well under way.

Retinopathy of Prematurity. This disorder, also called retrolental fibroplasia (RLF), occurs in premature infants exposed for prolonged periods to high levels of oxygen. In recent years, as the survival rate of preterm infants increased with improved management, the incidence of RLF has also increased.<sup>56</sup> One hope for reducing the incidence of ocular vascular maldevelopment in susceptible infants who require high levels of oxygen to sustain life because of cardiac and pulmonary complications of prematurity is to provide the infant with pharmacologic agents that will afford antioxidant protection.

It has been hypothesized that lipid peroxidation is the means by which oxygen induced photoreceptor degeneration occurs; increased fragility of retinal membranes, as in other biological systems, may be related to increased lipid peroxidation. For example, investigators at the University of California, Santa Cruz, have reported that purified preparations of rod outer segments show an increase in membrane fragility in the presence of oxygen. The products formed appear to be correlated with oxidative damage to polyunsaturated fatty acids in the membranes and to lipid oxidation. They also report, however, that the addition of vitamin E to the rod outer segment preparation resulted in a reduced susceptibility to oxygen damage.<sup>57</sup> It is reasonable to believe that vitamin E may be a pharmacologic agent that can provide anti-oxidant protection to the eyes of the preterm infant in addition to the multiple biological defenses against oxygen toxicity, such as superoxidedismutase, catalases, and heme-containing peroxidases.

Clinical researchers in Philadelphia (Pennsylvania Hospital, the Hospital of the University of Pennsylvania, and Children's Hospital of Philadelphia) have noted that vitamin E plays a central role in the regulation of intermediary metabolism and is a key biologic antioxidant; it may obviate the deleterious effects of high oxygen levels by binding free oxygen radicals. The small premature infant has very low levels of this nutrient. Since, in preliminary pilot studies and in a study of kittens,<sup>58</sup> vitamin E was found to provide some protection from excess oxygen, a randomized controlled clinical trial of this therapy has been initiated by this group after close collaboration with NEI staff and consultants in the preparation of the study design and protocols.<sup>59</sup>

## Uveitis .

Inflammatory disorders of the retina and choroid, commonly called uveitis, comprise a large group of highly destructive diseases: they are frequently blinding and in some cases painful. Characterized by the accumulation of inflammatory cells and fluid (edema), these diseases commonly affect the vitreous, the ciliary body, and the iris, in addition to the retina and choroid. Macular degeneration, glaucoma, and cataract may result as sequelae.

Uveitis may occur as a result of bacterial, viral, fungal, or parasitic infection, or as a result of an immunologic insult. It was estimated in 1972 that approximately 67,000 Americans suffered from the effects of severe uveitis. Of these, 23,000 were legally blind. It was also estimated that a much larger number of individuals suffered with less severe inflammatory disorders.<sup>1</sup>

The major objectives of NEI-supported research on uveitis have been to define the etiology and pathological processes of these disorders and to improve their diagnosis and management. An additional major goal has been to define the requirements for their prevention.

Patients with uveitis have been shown to have elevated levels of immune complexes.<sup>60</sup> For example, researchers at the University of California at San Francisco have recently provided preliminary evidence to indicate that patients with diffuse uveitis have significantly high levels of immune complexes, with IgG the predominant immunoglobulin.<sup>61</sup> Studies are continuing along these lines so as to characterize further these immune complexes and to examine their relationship to the disease. It is hoped that these studies will increase our understanding of the basic immunopathology of uveitis and will lead to the development of clinically useful immunodiagnostic assays.

Other investigators are using animal models in the quest for further understanding the immunological processes in uveitis. For example, scientists at the University of California at Los Angeles<sup>62</sup> and at the University of Louisville<sup>63</sup> have developed an experimentally induced autoimmune allergic uveitis in the guinea pig. The goals of such studies are to define the pathogenic mechanisms which come to play in the autoimmune forms of ocular inflammation in which the eye reacts to its own tissues and to develop methods for reversal of their devastating effects.

Animal models are also being developed for the study of uveitis resulting from specific infections. For example, investigators at Johns Hopkins University have developed virus-induced ocular inflammation in rats.<sup>64</sup> They are currently following the natural history of the retinopathy which occurs with this induced uveitis. Similarly, investigators at the University of California at San Francisco are studying uveitis induced by herpes virus,<sup>65</sup> by toxoplasmosis,<sup>66</sup> and by bacterial endophthalmitis.<sup>67</sup> At the University of Southern California, scientists

are developing and following an experimental monkey model of presumed ocular histoplasmic choroiditis.<sup>68</sup> The objectives of these studies are to identify the mechanisms by which the toxic agent penetrates cells in the uvea, to investigate the immunologic defense reactions evoked in the host, and to develop and evaluate rational therapies for treatment and for the prevention of recurrences.

### Workshops

Modern practice of ophthalmology has a greater dependency upon and use of diagnostic instrumentation. Improved technology over the span of more than 130 years has contributed to the development of a variety of clinical instruments which include direct and indirect ophthalmoscopes, biomicroscopes (slit lamps), operating microscopes, surgical lamps, and surgical lasers. There is need for an awareness of the potential hazard to the retina from combined thermal and direct phototoxic effects of light delivered to the optical media and fundus of the eye in the course of diagnostic and therapeutic procedures followed in normal clinical practice.<sup>1</sup>

In cooperation with the National Research Council-Committee on Vision of the National Academy of Sciences, a workshop, tentatively entitled "Role of Light in Clinical Practice," is being planned by the NEI. The purpose of this workshop would be to review experimental data which serve as a basis for clinical safety standards for exposure to light from ophthalmic instruments and to suggest directions for future research on potential hazards and their acute and chronic effects. The major topics would cover clinical disorders which may be affected by radiant energy, types of radiant energy commonly used in clinical practice, and research on the assessment of light effects.

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## CORNEAL DISEASES

### Introduction

Diseases of the cornea constitute an important cause of blindness and visual impairment and an immense economic burden on society. Over 2 million cases of corneal disorders and diseases occur each year in the United States. These constitute 62 percent of the total incidence of all acute and chronic disorders, diseases and injuries to the eye. Ocular infections and allergies (most of which are conjunctival) and ocular injuries (most of which result from foreign body or traumatic injuries to the cornea) constitute the bulk of these eye problems.

The Corneal Diseases program of the National Eye Institute provides research support for the investigation of disorders of the cornea, lids, conjunctiva, and the lacrimal gland, diseases of the orbit and external eye, and refractive errors and injuries of the cornea. The Corneal Diseases panel of the National Advisory Eye Council's Program Planning Subcommittee in its 1977 report, Vision Research--A National Plan: 1978-1982, has divided the program into the following six subprograms: (1) External Infections and Inflammatory Diseases; (2) Dry Eyes and Tear Abnormalities, Epithelial Disorders, and Drug Delivery; (3) Refractive Problems and Contact Lenses; (4) Corneal Edema, Dystrophies, and Inherited Disorders; (5) Corneal Transplantation and Stromal Injury and Repair; and (6) Tumors and other Lid, Conjunctival, and Orbital problems.

The following topics have been highlighted for discussion this year because of the important issues they address in relation to the research priorities identified in the Council's Plan. Since these research areas have either never been reviewed previously or only briefly referred to in prior annual reports, the evolutionary development of each topic will be traced from early scientific findings to present day research reports.

### Autoimmune Phenomena Associated with Cornea

Autoimmune phenomena are the immune responses of an individual to antigens which are normally present in his or her own body. They are thought to play a significant role in the initiation and propagation of several eye diseases. Such hypersensitivity to ocular tissues exists in the pathogenesis of phacogenic uveitis (lens); sympathetic ophthalmia (uvea); Vogt-Koyanagi-Harada syndrome (uvea, retina); Sjögren's syndrome (lacrimal glands); and probably endogenous uveitis (uvea, retina).

Evidence for autoantigenicity of the cornea and certain other ocular tissues, however, has not been documented. The cornea, like the lens, is formed early in the embryological development of the eye and is similarly avascular after maturation except for the limbal region. However, in comparison to the lens, the cornea has only limited organ specificity even though its properties are believed to result from avascularity and early isolation. It was noted by Manski and Martinez<sup>1</sup> in 1968 that antiserum to human cornea formed weak precipitin reactions when tested with other mammalian corneas (except from primates)

and responded negatively with corneas from other vertebrate classes. Studies by Whiteside et al<sup>2</sup> on soluble antigens derived from individual bovine corneal layers and tested with bovine serum-absorbed antisera demonstrated that each corneal layer, in addition to shared antigens, contained one or more "layer-specific" antigens. The corneal epithelium was considered to be the most strongly antigenic of the corneal layers, and the stroma proved to be the layer which contained most of the commonly shared corneal specific antigens present in this ocular tissue. Hall and coworkers<sup>3</sup> confirmed through the use of immunochemical techniques an earlier finding by Holt and Kinoshita<sup>4</sup> that a single nonserum protein comprised 40% of the soluble proteins of the corneal epithelium and appeared to be intrinsically corneal in nature. This protein was also found to be present in the stroma. Recently, Sun<sup>5</sup> has found corneal epithelial-specific proteins in cultures of rabbit cells distinct from those derived from epidermis and is in the process of purifying the former proteins from confluent cultures.

Antigens which may potentially have broader implications in regard to ophthalmic disease are those which reside on the surface of the cells in each of the corneal layers. Two types of such antigens were identified by Manski and Whiteside<sup>6,7</sup> in 1974. Metabolically dependent antigens (MDA) occur on the surface of regenerating or cultured cells that are in the state of active metabolism or regeneration. The other type of corneal membrane antigen is present on the cell surface independent of the metabolic state of the cell, that is, on excised or resting cells. Examples of the latter type antigens are Forssman and blood group antigens. The occurrence of the MDA antigens also appears to be a general biological phenomenon since they have been observed in other tissues.<sup>7</sup> Common MDA antigens are restricted to tissues of common embryological origin. By proper absorption with antisera, MDA antigens were also found to be of restricted tissue specificity. The metabolically independent cell surface antigens, on the other hand, appear to be rather universally distributed, some cross ontogenic and even species barriers.

It is possible that ocular involvement in diseases known to be autoimmune in nature may involve those MDA antigens of common ontogeny in the tissue primarily involved in the systemic disease and in ocular tissue. Autosensitization to tissues of meso- or ectodermal origin, even at a subclinical state, may also be a factor in corneal graft rejection. Autoantibodies to corneal and conjunctival epithelium have been detected by Brown and coworkers in the serum of patients suffering from Mooren's ulcers.<sup>8</sup> In addition, these workers have also recently demonstrated immunoglobulins and complement bound to the conjunctival epithelium of these patients.<sup>9</sup>

In summary, most investigations of autoimmune phenomena associated with the cornea have been concerned with detection of autoantibodies. It is reasonable to expect that future investigations in this area will be directed at cellular responses to autoimmune phenomena which may open new diagnostic and therapeutic avenues for research.

#### Treatment of Ocular Viral Infection-Recurrent HSV Infection

Herpetic simplex keratitis, which is the most common severe ocular viral infection in the United States, has an estimated incidence of 297,000 cases

annually. The disease typically begins during childhood and frequently recurs, causing severe visual disability and major loss in productivity. One of the most important recommendations of the Corneal Diseases panel of the National Advisory Eye Council's Program Planning Subcommittee is the development of new antiviral therapy for herpes simplex keratitis. Approximately 50% of those who develop herpes simplex epithelial keratitis are likely to experience recurrence of epithelial disease or a complication of deep stromal keratitis or uveitis within two years.<sup>10</sup>

The herpes simplex virus can be isolated from 75% of untreated patients with active dendritic keratitis,<sup>11</sup> and tear film cultures are periodically positive, whether or not there is evidence of clinical disease. Although the latent virus was originally thought to reside in the lacrimal gland, Nesburn and coworkers<sup>10</sup> reported in 1972 that the trigeminal ganglion, and perhaps other ganglia around the eye, may be a source of virus for reinfection.<sup>12</sup>

Hopes for pharmacological treatment of herpetic keratitis were raised in 1962 with the first successful application by Kaufman et al of an antiviral drug, idoxuridine (IDU), to the treatment of an ocular viral disease.<sup>13</sup> It was soon found, however, that although this drug was somewhat effective in controlling the disease if treatment was initiated early, IDU alone could not prevent recurrences of the epithelial disease.<sup>10</sup> In addition, further research revealed that IDU was chemically unstable and produced drug allergies and toxicity when used topically. Because of continuing problems associated with IDU, new antiviral agents were subsequently investigated. Adenine arabinoside (Ara-A, vidarabine) which functions like IDU in inhibiting DNA synthesis in infection-producing viruses was found to be equally effective to IDU and has better solubility and lower toxicity, and is effective in cases where resistance has been found to the latter drug.<sup>14</sup> Another drug, trifluorothymidine, was found to be even more potent and active than IDU in the topical treatment of rabbit herpes keratitis<sup>15</sup> and was found to be more effective than Ara-A in the treatment of superficial herpetic keratitis.<sup>16</sup> The former compound was found to be more effective than Ara-A in the treatment of stromal disease because of its solubility and high activity, but it is not readily available for clinical use due to low pharmaceutical production. This drug, like IDU, when given to rabbits who were developing recurrences chronically, was ineffective in preventing recurrences though the lesions were of smaller size and healed rapidly.<sup>15</sup>

Interferon, a protein produced by lymphocytes, in response to active herpetic infection, has been shown in low concentration to have some nonimmunological protective effect against vaccinia and other kinds of viral keratitis in the rabbit. The protein is species specific, and only human or primate interferon can be expected to be used with any degree of success in human herpetic keratitis. The use of topical rabbit interferon in rabbits and topical human leukocyte interferon in the owl monkey is effective in protecting corneas from a challenge infection with the virus.<sup>17</sup> At the present time, high-titered human leukocyte interferon is being used in clinical investigations supported jointly by NEI and the National Institute of Allergy and Infectious Diseases to determine whether the purified biological compound can decrease or prevent recurrences of herpetic corneal infection.<sup>18</sup> Because homologous interferon has been difficult and expensive to obtain, other studies have been pursued which

attempt to stimulate interferon levels in vivo through use of chemical inducers. Park and Baron<sup>19</sup> demonstrated in 1968 that poly I:C, a double-stranded synthetic RNA, was capable of stimulating interferon production and had both a therapeutic and prophylactic effect on herpes simplex keratitis in the rabbit. The therapeutic effect, however, was found to be of short duration, but the prophylactic effects in rabbits could completely prevent recurrences of the disease.<sup>20</sup>

When topical poly I:C was studied in man, it was found that, unlike what occurs in the rabbit, only small amounts of interferon were produced in the tears for only about 48 hours.<sup>21</sup> Similar results were obtained when the systemic route was used for the drug in humans. Other interferon inducers have been examined but have not proven to be of clinical use.<sup>22</sup>

Other antiviral agents have been tested on animal models with various degrees of success. Phosphonoacetic acid has recently been reported to be as effective as IDU in treatment of the rabbit herpetic disease, but further studies must be done to evaluate its full potential.<sup>23</sup> Adenine arabinoside monophosphate (Ara-AMP) is a more soluble derivative of Ara-A and has been demonstrated to have antiviral activity equivalent to Ara-A against herpes type 1 and 2 in tissue culture<sup>24</sup> and against type 1 epithelial keratitis in rabbits.<sup>24,25</sup> Recently, another analogue of thymidine has been used by Albert and coworkers<sup>26</sup> to treat experimental rabbit keratitis. The drug 5-Iodo-5' amino 2' 5'-dideoxyuridine (AIU) was found to be somewhat less effective than IDU, but the drug's lack of cellular toxicity may make it a promising new agent.

Thus, vision researchers have taken a leading role in the development, evaluation, and delivery of antiviral agents. Some of the advances in vision research in this area have been translated to treatment of other viral infections. The use of Ara-A for effective treatment of herpes encephalitis is such an example.

### Corneal Transplantation

For many years, it was accepted that the high rate of success of corneal grafts in relation to grafts of other types of tissue was not so much due to the fact that the cornea had few cells and was antigenically weak, but rather that it was placed in a privileged avascular site. Later, it was shown that transplanted corneal tissue did not have to be placed in a vascularized bed to sensitize a host since graft rejections could also occur with avascular corneas.<sup>27</sup> This observation led to a search for both cellular and noncellular antigens in this tissue.

Ehlers and Ahrons,<sup>28</sup> in 1971, showed that histocompatibility antigens are present on the surface of corneal cells and postulated that these antigens may play a role in allograft rejection as had been previously determined for kidney transplantation. This observation was confirmed by Stark and coworkers,<sup>29</sup> and Newsome and coworkers,<sup>30</sup> using cells from all three layers found in the cornea. Cultured corneal cells from these cell populations, and lymphocytes from the same donors were found to be concordant in histocompatibility typing.

Histocompatibility antigens in humans reside on the two number 6

chromosomes, each of which has histocompatibility zones A and B and phenotypes which consist of two antigens from series A and two from series B. Allansmith et al<sup>31</sup> in a study of 43 nonselected corneal donor-recipient pairs found that matching for two to four such antigens did not seem to correlate with the fate of the graft. However, Vannis<sup>32</sup> has reported that matching for four antigens shows improved results and suggests that such matching be done in conjunction with immunosuppressive therapy.

The production of humoral antibodies against HLA antigens in patients with vascularized corneal beds and immunological rejection of allografts was also demonstrated by Stark and coworkers.<sup>29</sup> Stark<sup>33</sup> has extended these observations and is presently engaged in a randomized clinical trial to determine the influence of recipient presensitization against HLA antigens of the donor on the outcome of corneal transplantation.

In related studies, Vidal and Polack<sup>34</sup> have studied the fate of experimental corneal grafts after sensitization with a streptococcal group A antigen. These workers found if normal rabbits were exposed to this antigen and if corneal transplants were similarly exposed to the antigen before keratoplasty, severe graft reactions developed two months after keratoplasty in the sensitized animals, but not in the control animals. These observations suggest the possibility that homograft reactions could be triggered by bacterial antigens which are capable of crossreacting with certain histocompatibility antigens and rejection of the graft results through reaction with these histocompatibility antigens.

The Corneal Diseases panel in Vision Research--A National Plan: 1978-1982 identified corneal transplantation as a priority area for NEI-supported research because recent progress has been rapid and good further progress can be expected with a small investment of funds and manpower. The cornea can serve as a simplified model for identifying possibilities for therapy which may be overlooked in the more complex rejection phenomena associated with kidney or heart transplants.

#### Corneal Epithelial Wound Healing

The healing of epithelial wounds was shown by Friedenwald and Buschke<sup>35</sup> in 1944 to be accomplished primarily by a migration of neighboring epithelial cells and only secondarily by cell division in the case of nonpenetrating wounds. Kuwabara et al<sup>36</sup> have recently shown that the corneal epithelial cells have a unique sliding ability. The epithelial cells spread and migrate in an amoebic fashion without mitotic activity when the continuity of the epithelial layer is broken, and prompt sliding for sealing the wound defect is apparently the first step in wound healing of the superficial cornea. Gipson and Anderson<sup>37</sup> have recently demonstrated in organ-cultured rat corneas with small central corneal epithelial abrasions that incubation with concanavalin A at 100 µg/ml was able to stop the migration of cells on the ocular surface. This lectin binds selectively to glucose and mannose molecules, and microscopy of the lectin conjugated to ferritin indicated an increase of these sugar molecules on cell membranes of migrating as compared to normal cells.

With large wounds that cannot be completely covered by the initial

migration, the deficit in cell number is made up by a diminished rate of desquamation, and mitotic activity may never reach a higher level than normal. Even so, the mitosis that does exist is found not at the wound margin or in the migrating cells, but in the undisturbed epithelium some distance from the wound. This has been confirmed autoradiographically with  $^3\text{[H]}$ -thymidine by Hanna and coworkers,<sup>38</sup> in 1961, which reveals that the potential proliferative activity is greater at that site than required for the normal epithelial renewal rate of seven days.

Dunnington and Smelser in 1958 observed that incised wounds of rabbit corneas are completely covered by glycogen-filled epithelial cells by 24 hours.<sup>39</sup> In the first electron microscopic study of nonpenetrating corneal incisions in the rabbit eye, LaTessa and Ross<sup>40</sup> in 1964 observed that epithelium extended into the wound area by 1 1/2 hours, with complete epithelization in 24 to 36 hours. More recently, Pfister and Burnstein have described the early epithelial regrowth rates after alkali burns of the cornea.<sup>41</sup> After 6mm and 12mm alkali burns, the regrowth rate was found to be similar to that found after simple abrasions, but twice that found after keratectomy. Simultaneous keratectomy and burning of corneas showed the same time of epithelial reversal.

If the whole corneal covering is removed, Friedenwald<sup>42</sup> noted in 1951 that the denuded surface becomes covered by epithelium of conjunctival origin within one week, the new layer being one cell layer thick and containing conjunctival goblet cells. By the end of six weeks, the covering was thickened into several layers and has undergone metaphasia into cells indistinguishable from normal corneal epithelium. This transformation suggests that the stroma has an inductive influence. Growth factors, specifically the epidermal growth factor (EGF), have been examined for their effect on the regeneration of corneal epithelium. Savage and Cohen<sup>43</sup> demonstrated in 1973 that EGF stimulates the proliferation of corneal epithelium both in organ culture and in vivo. The topical application of EGF to the eyes of rabbits with corneal wounds resulted in a marked hyperplasia. However, the effect noted in vivo was transient, and the epithelium rapidly regressed to its normal thickness, whereas the studies in vitro were irreversible. This has led to recent suggestion by Gospodarowicz<sup>44</sup> that EGF may play a role in the regeneration of epithelium after injury.

More investigation is needed to determine what growth factors can stimulate cell division in the corneal epithelium. Cell biologists and experimental pathologists should be recruited and trained for research efforts in epithelial wound healing.

### Comparisons Between Corneal and Conjunctival Epithelium

Corneal and conjunctival epithelium play similar roles for the eye in providing protection from infection for the underlying tissues and acting as a sensory alert to ocular trauma. Histologically and biochemically, however, these two cell layers are quite different. The conjunctival epithelium is thinner and its cells are more loosely packed with fewer desmosomes and hemidesmosomes than is the case for corneal epithelium. A more striking histologic difference, however, is the presence of mucin-producing goblet cells and a direct blood supply in the conjunctival epithelium which is totally

lacking in the corneal epithelium.

Metabolically, in the conjunctival epithelium, there is a high rate of activity of glycolytic, tricarboxylic acid cycle and respiratory chain enzymes but relatively low activity for the hexose monophosphate shunt pathway.<sup>45</sup> By contrast, Kinoshita and coworkers<sup>46</sup> found earlier in 1955 that corneal epithelium has a high level of hexose monophosphate shunt activity and a relatively sluggish tricarboxylic acid cycle.<sup>46</sup> Conjunctival epithelium is capable of transforming to corneal epithelium for purposes of regeneration over denuded stroma, but the former tissue layer must undergo significant biochemical changes. Thoft and Friend<sup>47</sup> have demonstrated that although this cellular transformation appears to be complete histologically within six weeks, regenerating epithelium of conjunctival origin has reduced glycogen content and lowered lactic dehydrogenase levels as compared to that of corneal origin. Such biochemical alterations may have a direct effect on epithelial integrity during the critical stage of wound healing and protection of tissue from proteolytic enzymes. These workers<sup>48</sup> also observed substantially lower healing rates for conjunctivally derived epithelium growing over the denuded surface as well as decreased stromal wound strength than for corneally derived epithelium. Kuwabara et al<sup>36</sup> also found slower healing rates for conjunctival epithelium and was able to retard the sliding of such epithelial cells into wounds through depletion of glycogen in vitro by treatment with amylase.

In its 1977 report, the National Advisory Eye Council identified the need for expanded research on the immunological aspects of ocular diseases and for application of the concepts from recent advances in the field of immunology to the study of the visual system. The former objective has been approached through the issuance in August 1978 of an NEI request for a grant application announcement entitled "Immunological Aspects of Ocular Disease." In addition, an international symposium,<sup>49</sup> "Immunology and Immunopathology of the Eye" was held in May 1978 in San Francisco, and papers were presented by approximately 80 scientists from around the world engaged in various aspects of ocular immunology and vision research. Topics discussed were immunogenetics, transplantation biology, tumor immunology, immunopathology of uveitis, herpetic keratouveitis, and a variety of subjects of general interest in immunology of the eye.

The transfer of knowledge from other fields of immunology to vision research will be stimulated through the scheduling of a meeting in late August 1978 between the NEI and NIAID staff for the purpose of planning three immunological workshops on specified topics in ocular immunology. Subjects identified for such consideration are: (1) immunogenetics and transplantation immunity; (2) autoimmune phenomena and ocular disorders; and (3) infection, inflammation and allergy. Nonvision research scientists will be invited and interspersed with those engaged in ophthalmic immunology to assess the state of the art in each of these research areas and make recommendations for encouraging research in the most promising areas.

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## CATARACT

Opacification of the lens is a common disability affecting large numbers of otherwise healthy people. This progressively disabling affliction ultimately results in blindness with great costs to the individual and to society. Each year in the United States, there are approximately 400,000 operations performed to remove cataract, and it is estimated that 1,670,000 Americans have vision difficulties because of developing cataract. Means are being sought for its prevention, for improvements in its amelioration, and for its cure.

### Cooperative Cataract Research Group (CCRG)

This group of cataract investigators was formed in 1976 with the specific objective of applying their diverse expertise to the establishment of the fundamental morphological, biochemical, physiological, and biophysical characteristics of the normal human lens. This information will, in turn, provide a baseline for changes that occur during the process of cataractogenesis. A series of workshops has been held to standardize procedures for collaborative studies, and several studies are now actively in progress.

One major baseline study has been completed by the CCRG and has been published. In this, a system is proposed<sup>1</sup> which permits classification of the lens opacity immediately after extraction and which provides for a classification that is independent of clinical specifications. The system, which involves photographic recording, and thus permits reconsideration of the original observations if this is required, makes use of stereoscopic photography. This system has been referred to as the "basic classification scheme." An "extended classification scheme" is envisioned as a next development. In this, there will be in vivo slit lamp photography and quantification of light absorption and scatter. These studies are in keeping with the recommendations of the National Advisory Eye Council.

Using the American CCRG as a model, two additional CCRGs have been established. One includes membership from several western European countries. This group has been founded and is already in active operation. The second, in Japan, is in the process of program development. It is expected that cooperative arrangements will ensue among the three groups.

### Diet Deficiency Cataract

Tryptophan deficiency in the diet produces a reproducible cataract in guinea pigs and rats which continues to attract the attention of investigators. Weanling rats grown on a low tryptophan diet develop lens changes at three to four weeks which first appear as an inner reflecting surface within the lens.<sup>2</sup> At this time there is widening of the lens bow suggesting abnormal maturation of fiber cells. Later, the nuclear zone becomes hazy, but it does not develop into a dense nuclear cataract. Tissue analysis indicates a significant reduction in Na/K-ATP-ase activity but no change in glutathione content. Also, as with cataracts produced by x-ray irradiation or high galactose diet, there is reduction in the crystallin fraction of lens protein.<sup>3</sup> This animal model is regarded favorably for study of abnormalities and growth of the lens which may

provide clues to changes occurring in senile cataract in man, an area designated as high priority by the National Advisory Eye Council in its report, Vision Research - A National Plan: 1978-1982. Future studies will be directed both at the effect of tryptophan deficiency on the process of cellular proliferation and specialization, as well as on changes in cellular composition and biochemical function.

### Contact Adhesion

Intraocular lenses destroy endothelial cells by contact adhesion between the acrylic lens and endothelial surfaces during cataract surgery.<sup>4</sup> Because endothelial cells are essentially nonregenerative in man, are progressively depleted with age, and are subject to clarity loss after damage, such damage is a serious medical concern. The phenomenon of cell damage appears related to hydrophobic interactions between the polymethyl methacrylate polymer, of which the acrylic lens is composed, and cell surfaces in the corneal endothelium. It has been found that adhesion damage to endothelial cells can be completely eliminated by application of a hydrophilic polymer such as polyvinylpyrrolidone (PVP).<sup>4</sup> It is noteworthy that contact adhesion-produced tissue damage appears to be a more general phenomenon than has previously been realized and can occur as a result of even very brief contact between exposed tissues and such foreign materials as plastics, rubber, metal, and glass. Thus, surgical gloves, surgical instruments, and catheter surfaces, if not properly pretreated with hydrophilic polymer, may be the cause of tissue damage-connected postsurgical complications. The findings from these NEI-supported studies will be of considerable interest to surgical medicine in general.

### Lens Proteins

Identification and localization of lens proteins in the normal lens and changes that occur with cataract formation represent important continuing aspects of eye research. Through the application of immunological techniques, Maisel<sup>5</sup> has shown that the delta crystallin which comprises 38% of the membrane fraction of normal chick lens outer cortex occurs in several immunologically identical subunits ranging in molecular weight from 43,000 to 48,000 daltons. In the cell nucleus membrane, only a single subunit of 43,000 daltons appears. The soluble protein fraction from normal human, bovine, and rabbit lenses separate on Sepharose into five sharply defined peaks, corresponding to the crystallins,  $\text{HM}_\alpha$ ,  $\beta_{\text{H}}$ ,  $\beta_{\text{L}}$ , and Y. The corresponding fraction taken from cataractous human lens gives broadened and overlapping peaks which indicates that changes have taken place in the composition of these proteins.<sup>6</sup> Determination of the nature of these changes may provide clues to biochemical steps responsible for cataract formation.

### Congenital Disorders

Among the 49,000 American cases of newly diagnosed congenital eye disorders reported each year, a large proportion have cataract. Induced cataracts in laboratory animals have provided useful models for sugar, traumatic, and other cataract types to which man is subject. However, naturally occurring cataract types have received much less attention because of a general paucity of animal models of relevant spontaneously occurring cataract, although studies in animal models have been recommended by the National Advisory Eye Council.

Several mutant animal strains that develop spontaneous cataracts are now coming under active study.

An x-ray-induced rat mutation appears to provide a model for human congenital anterior polar cataract. In this model, retinal folding is observed during gestation. This is believed to exert a pressure on the lens stalk that results in hyperplasia, which in turn is followed by the formation of the anterior polar cataract. Age-related cataracts in these animals appear to be correlated with the presence of acid phosphatase extracellularly in the lens and also with an increase in the smooth endoplasmic reticulum which is regarded as the source of the enzyme. Development of cataract is accompanied by conversion of cysteine sulphydryl to cystine disulfide with the formation of high molecular weight protein aggregates.<sup>7</sup>

Histological and biochemical studies have been undertaken on congenital cataracts in miniature schnauzers. Morphological and ultrastructural analyses of eye development will be made in a highly inbred strain on anophthalmic and microphthalmic mice, and molecular biological techniques for analyzing differences in the regulation of genetic information in normal, genetically altered lenses leading to cataract will be applied in studies of Nakano mutant mouse lens cells.

Marfan's syndrome is an inherited autosomal dominant disorder of the connective tissue that results in dislocation of the lens. Scanning electron microscopic studies of diseased human lenses suggest that the basic defect lies in structural proteins that play an integral role both in zonular and capsular construction.<sup>8</sup> Because the component proteins in the two regions are not identical, it is speculated that they may share a common genetically derived defect in a protein component which may be common to the two regions. Determination of the composition of the structural proteins in these tissues has been recommended by the National Advisory Eye Council. Such information should provide further insight into the basis for the disorder.

Aphakia, an autosomal recessive single gene mutation in the mouse, seriously affects the development of the ocular lens. Up to advanced stages of lens invagination, morphogenesis proceeds normally. In the late lens cup and early lens vesicle stage, the epithelium of the lens rudiment becomes disorganized and the lumen of the vesicle becomes filled with rounded cells. Aphakic mice embryos show significantly greater numbers of maloriented mitoses in the lens placode and early lens cup than do normal embryos, and it is suggested that cellular release may be a consequence of such malorientation. In addition, aphakic mice fail to develop crystallins, so that the genetic defect exerts both morphological and biochemical consequences.<sup>9</sup> It would appear that regulation of the normal plane of cell division may be a requirement for subsequent orderly development of the lens. Identification of the factors involved in abnormal lens development in laboratory animals should provide clues to the corresponding disorder in humans.

### Diagnosis

It had been shown earlier using laser light scattering spectroscopy that a decrease in protein diffusivity in whole excised lenses is associated with some types of nuclear cataract in humans. Use of this technique has now been

applied in vivo to measurement of protein diffusivity in rabbit lenses. At levels of laser light irradiation considered to be safe in humans, changes in protein diffusivity could be observed and quantitated at a stage before opacity was detectable by visual inspection with an ophthalmoscope or a slit lamp microscope.<sup>10</sup>

This technique offers potential advantages of greater sensitivity in studies of the effects of aging, drugs, diet, and other cataractous factors on cataract formation in laboratory animals as well as of earlier diagnosis for some types of cataract in humans. In addition, it provides a degree of selectivity, because opacity may result from a variety of inhomogeneities within the lens, whereas protein diffusivity concerns only the random motion of the proteins present. Improved means for diagnosis and detection should aid in determination of the mechanisms of cataract production, a high priority in the NEI program.

### Molecular Aspects of Cataractogenesis

Progress has been made during the past decade in understanding lens differentiation, growth, and metabolism, on how the molecular structure is preserved and on how disruption of these processes causes cataract formation. Continued study in these areas is of prime importance. Present evidence points to multiple molecular mechanisms for cataract formation in the human lens. By study of the mechanisms and features of diverse models of animal cataracts, we hope to gain increasing insight into the process of cataractogenesis and so help to broaden our understanding of the human disorder.

Glucocorticoids are known to induce posterior subcapsular cataract in man following either topical or systemic administration. However, the locus of primary action until now has remained unspecified. Southren has now demonstrated that lens epithelium in the calf contains a specific receptor for glucocorticoid binding.<sup>11</sup> These results suggest that this class of steroids may play a direct physiological role in lens differentiation. Further, since cataract formation is observed with high doses of the steroids, it is suggested that this may be the result of abnormal patterns of cell differentiation.

Cold cataract, the opacity that appears upon lowering eye temperature, has been the subject of considerable study particularly since it is a reproducible and reversible phenomenon that can be studied in the excised lens. Earlier studies indicated that this form of cataract is associated with changes in the physiochemical state of lens protein, but the nature of the changes has remained obscure. Light scattering studies have suggested phase separation of a protein-water binary mixture as the cause of opacity. This suggestion has received significant support from recent studies which demonstrate a close parallel between cold cataract in the lens and the appearance of a temperature-dependent reversible opacity in lysozyme-salt water mixtures.<sup>12</sup>

Earlier studies have indicated an increase in protein molecular weight in the aging lens which is believed to be related to certain types of senile cataract formation. With aging there is also a marked increase in nontryptophan protein-bound fluorescence which appears primarily associated with high molecular weight fractions. Studies of the phenomenon have been undertaken with the

objective of gaining insight into the chemistry of the process leading to increased protein molecular weight. It is now apparent that the fluorescence is associated with a complex group of chemical structures. Identified so far are a beta carboline, anthranilic acid and bityrosine.<sup>13</sup> It is suggested that bityrosine may be involved as a cross-linking agent in old and cataractous lenses.

Raman spectroscopy provides a noninvasive, nondestructive probe of chemical structure that may be applied directly to living lens tissues. This tool has now been used to investigate the conversion of protein chain thiol groups to interchain disulfides, a process long believed to be involved in protein molecular weight increases associated with aging and senile cataract. Since change in thiol levels may result not only from conversion to disulfide but also from changes in protein composition, the ratio of tyrosine to phenylalanine was monitored as a measure of change in protein composition.<sup>14</sup> The Raman spectral results show clearly that virtually all sulfhydryl groups in the lens nucleus are converted to disulfide as the rat or mouse ages. In the lens cortex there is an increase in the ratio of alpha to gamma crystallin and a decrease in thiol with concomitant but not equivalent increase in disulfide. These studies provide important support for prior reports of thiol to disulfide conversion, which is especially important because of technical difficulties that are associated with the usual analytical methods that have been used.

#### Development of a New Cataract Surgical Tool

The National Eye Institute and the National Aeronautics and Space Administration signed a cooperative agreement to conduct laboratory and clinical tests of a new surgical instrument for the removal of hard cataracts.

The new instrument, developed by NASA's Lewis Research Center, Cleveland, Ohio, consists of a surgical hand piece, a regulated flow system for infusion fluid, and a peristaltic outflow pump. This instrument requires only a small opening in the cornea and is designed to remove cataracts of all degrees of hardness.

The NEI/NASA program involves refinement of surgical techniques with the cataract instrument and the design and implementation of preliminary clinical trials. The Eye Research Institute of Retina Foundation, Boston, is participating in the project by evaluating the instrument and developing surgical procedures. ERI surgeons have already performed limited trials in India and in the Phillippines in conjunction with surgeons of those countries.

Successful development of an instrument that can remove hard cataracts quickly through a small opening in the cornea would have significant impact on the delivery of eye care in some developing nations. In many of these countries, the numbers of people with hard, vision debilitating cataracts are extremely large and consume a relatively large portion of health care resources. The instrument is intended to reduce both surgical time and postoperative care with a consequent savings in manpower and other resources.

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## GLAUCOMA

The recent report of the National Advisory Eye Council, Vision Research-A National Plan: 1978-1982 summarizes the need for continuing research on the problems of glaucoma. In the United States, over one million people have impaired vision attributable to glaucoma, and about 178,000 new cases are diagnosed yearly; over 200,000 of these people have severely compromised vision, and over 50,000 of these are legally blind as consequences of glaucoma. Our society additionally pays a large price, financially and in lost productivity and time for diagnosis and treatment of the diseases classified as glaucoma. There is clear need for a vigorous ongoing research program into the etiology, natural history, diagnosis, and medical or surgical treatments of the disease. Of equal importance are studies that might lead to preventive or early prophylactic measures by means of predictive criteria based upon correlations with genetic markers, responses to provocative tests, quantitation of anatomic eye measurements and documentation of progressive changes in such measurements, and changes in physiologic and biophysical aspects of aqueous humor dynamics.

Glaucoma is a term used to define a group of ocular diseases generally first characterized by above-normal intraocular pressure (IOP), by visual field losses, and by changes in optic nervehead morphology. Occasionally, however, glaucomatous field losses are also seen in normotensive eyes. Not all ocular hypertensives have glaucoma, nor is it known how many such individuals may develop symptoms of the disease. Further research is required to refine the meaning of intraocular pressure measurements, particularly in defining the basis of inpatient variability of measurement (diurnal rhythms, circadian rhythms, dietary effects, hormonal effects, emotional effects, or other clinical problems). Major classes of glaucoma are defined as primary open-angle (POAG), acute angle-closure, congenital (perhaps having several etiologies), and a variety of secondary glaucomas developed as a consequence of other eye diseases. Certain of these diseases are readily treated by medical or surgical means; others are extremely difficult to manage. Some information describing ongoing and encouraging new research into prediction, diagnosis, and therapy of the glaucomas is presented in this report. The NEI has supported individual research projects dealing with these problems via the project grant, as well as larger-scale concerted approaches dedicated to developing optimal therapies for glaucoma by means of the Specialized Clinical Research Center and Core Center for Vision Research grants. A limited number of controlled clinical trials are also being supported.

During the past year, two grant announcements soliciting new research grant applications into glaucoma-related projects were published in the NIH Guide for Grants and Contracts (Vol. 7, Number 10, August 4, 1978). Hydrodynamics of the Eye emphasizes the need for research in the following areas: fundamental dynamics of aqueous formation and drainage and the relationships of IOP levels to both aspects of aqueous steady state balance, new drugs and mechanisms of action of drugs influencing secretion and outflow of aqueous, factors influencing the circadian variations of IOP in normal persons and glaucomatous individuals, and metabolism of anterior chamber tissues in normal and glaucomatous eyes. Secondary Glaucomas defines eight types of glaucoma occurring consequent to other conditions which are themselves serious diseases

and suggests some research approaches to an understanding of them. These diseases include: glaucoma secondary to uveitis, glaucoma following exfoliation syndrome, hemolytic or erthroclastic glaucoma, glaucoma secondary to soluble or particulate lens antigen release, neovascular glaucoma, glaucoma associated with pigment dispersion syndrome, glaucoma following invasion of the anterior chamber of corneal epithelium, and glaucoma associated with retinal detachment.

To support clinical research further, a new Glaucoma Clinical Research Center was established at the Massachusetts Eye and Ear Infirmary, and a Center for Vision Research was established in the Department of Ophthalmology of the University of California, San Francisco, to aid in basic and clinical research activities of the department. In addition, four controlled clinical trials are approaching implementation as Manuals of Procedure near completion.

### Intraocular Pressure Factors

The first question to be considered in dealing with ocular hypertension in the absence of other signs of glaucoma (visual field losses, optic disc changes) is whether or not to initiate medical treatment since ocular hypertension is not always predictive of glaucoma. One 42-month study involved 60 ocular hypertensive persons (pressures over 21mm Hg) given no therapy and 38 normal individuals. Intraocular pressures of control subjects showed no significant changes or directional trends with time; pressures of ocular hypertensives were either stable, showed cyclic up-down swings, or tended to increase. This latter group of patients may be the individuals liable to develop glaucoma.<sup>1</sup>

Retrospective studies of ocular hypertensives showed that of 75 persons with normal visual fields initially, 25 ultimately developed field defects;<sup>2</sup> also, the fate of 31 patients with binocular hypertension and monocular field losses were followed over a three to seven year period, and in 9 cases the fellow eyes developed glaucoma.<sup>3</sup> Prospective studies are attempting to define the predictive values of measurements of visual field measurements in ocular hypertensives.<sup>2</sup>

The influence of diurnal or circadian rhythms on intraocular pressures has been noted, as has the fact that corticosteroid therapy causes increased intraocular pressures and visual field changes characteristic of glaucoma. In detailed studies of the correlation of plasma cortisol levels with IOP, with hospitalized subjects on a stabilized regime, a midmorning peak of plasma cortisol concentration coincided with the daily peak of IOP and with a daily low in tonographic outflow facility. Significant differences in diurnal levels of plasma cortisol and IOP were observed between normal subjects and POAG patients, and for blind persons midmorning cortisol levels were below normal.<sup>4</sup> Other studies, by the same investigator, have indicated a role of the central nervous system and a disturbance of the pituitary-hypothalamic axis-steroid hormone system in controlling IOP in patients with glaucoma.<sup>5</sup>

### Visual Fields Factors and the Optic Disc Morphology

Glaucoma is characterized clinically not only by ocular hypertension, but by visual field changes and changes in the configuration and appearance of the optic cup. Several investigators are studying instruments for measuring and

recording these parameters to monitor patient progress.

A recently described, commercially produced static perimeter (Fieldmaster, Synamed, Inc.) allows rapid visual field measurements by inexperienced personnel with results highly compatible with those obtained by Goldmann perimetry, and an 80% to 98.5% detection rate (depending upon number of target luminescence values) with 4% false positive rate.<sup>6</sup> This instrument may have value in mass screening programs for singling out patients for further intensive studies.

Efforts continue to record precisely measured optic disc dimensions in order to define more exactly correlations of size and conformation with glaucomatous visual field losses and to enable an accurate record to be kept of progressive changes in a given eye. Development and refinement of instrumentation and methodology, especially for stereo photography of the optic disc using computer analysis for photogrammetric evaluation of disc dimensions, is ongoing. Thus, a number of investigators using adaptations of the basic Donaldson fundus camera and variously based computer programs to analyze disc photographs have further refined conditions for accurate measurements.<sup>7-10</sup> In one study, mean values for optic cup volumes and areas differed among ocular hypertensives, ocular hypertensives with disc defects, and glaucoma patients. However, overlaps among the groups were too large for measurements to be individually predictive, and inter-eye asymmetry appeared to be a more valid predictor of glaucomatous damage.<sup>8</sup> The effects of both technical variables in the use of the Donaldson camera and eye variables as affecting computer-generated optic disc dimensions were explored, and a densitometric method for measuring disc pallor was developed.<sup>9,10</sup>

#### Effects of Intraocular Pressure on Optic Nerve Function

Efforts continue to determine how increased IOP is related to the mechanism of optic nervehead damage. Measurements of rate of transport of labeled markers in the nerve (axonal transport) are one means of analyzing nerve function. Injection of autologous, fixed, red cell ghosts into the anterior chamber produced a chronic rise in IOP in rabbit eyes, and in monkeys also caused histologic changes in the optic disc and nervehead typical of glaucoma. Orthograde axonal transport studies, monitored by light and electron microscopy, indicated that immediate reversible axonal damage occurred, and that with long-term increased IOP, permanent axonal degeneration resulted. This appears to be a good model for hemorrhagic glaucoma (a secondary glaucoma).<sup>11</sup> In other studies, the effects of increased IOP on rapid axonal transport from the retinal ganglion to the lateral geniculate nucleus were followed: radioactively labeled protein increasingly accumulated with time at the lamina cribrosa, and the decreased rate of transport was not due to a general slowdown in all axons, but rather to complete blockade of certain axons.<sup>12</sup> In other studies, injection of red cells into human eyes (prior to enucleation for malignancies) raised IOP. Tissues obtained at subsequent intervals to five days were examined by light and electron microscopy, and numerous changes were observed in axonal, glial, and vascular elements of the optic nervehead.<sup>13</sup>

Ischemia in the region of the lamina cribrosa was considered as a possible cause for the reduced axonal transport accompanying increased intraocular pressure. A breakdown in the local blood-brain barrier in the optic nerve

was indicated by leakage of fluorescent dyes in laboratory animals having an increased IOP.<sup>14</sup>

Ischemia per se may not explain optic nerve degeneration with high IOP. In other studies, perfusion pressures and blood flow in retina and optic nerve were considered. At moderate increases in IOP there was little change of blood flow in the retina or the prelamellar part of the optic nerve, while at higher pressures there was a marked reduction in blood flow. After 30 minutes of sustained high IOP, flow in the lamina was constant. When very high pressures were reached, an increased blood flow was seen at the start of the retrolaminar part of the nerve. These observations suggested that a reduction of blood flow does not stop axoplasmic transport at the lamina cribrosa nor is retrolaminar degeneration simply due to a reduced blood supply.<sup>15</sup>

In rabbit eyes in vitro, the effects of optic nerve compression at various pressures indicated that nerve tissue metabolism was not significantly interrupted. Axonal transport of labeled protein was inhibited at 30mm Hg, and the degree of inhibition was increased in the presence of added sodium azide (inhibiting respiration) or colchicine (inhibiting microtubule function). The effects of moderate pressure on the nerve were reversible, while those of higher pressure were not.<sup>16</sup>

#### Aqueous Humor Dynamics

Steady state intraocular pressure is maintained by a balance between the forces controlling aqueous humor production and those influencing its outflow from the eye. Several studies have considered phenomena which may influence outflow. Tracers (red cells or horseradish peroxidase) were refluxed into Schlemm's canal in live monkey eyes which were fixed in vivo; also, eyes maintained at stated pressures were fixed in situ to define the status of outflow channels. The red cell studies showed that both the trabecular endothelium lining Schlemm's canal and the endothelial tubules distend at physiologic pressures and collapse and constrict at lower pressures; further, the endothelium of Schlemm's canal does not permit passage of red cells or plasma. At 25mm Hg, the endothelial lining extended far into Schlemm's canal, nearly obstructing it, while at 4mm Hg the trabecular meshwork was collapsed far from the external wall of the canal.<sup>17</sup>

The possibility that phacolytic glaucoma might be caused by blockage of drainage channels by lens particles or soluble high molecular weight protein aggregates leaked from cataractous lenses has been explored.<sup>18,19</sup> Aqueous humor was obtained at cataract surgery from patients with phacolytic glaucoma and from others with immature cataracts and normal IOP. Soluble proteins of aqueous obtained from phacolytic glaucoma patients were fractionated into two major classes, high molecular weight aggregates and a fraction, containing lower molecular weight proteins, which coincided with the only significant group of proteins obtained from the aqueous from eyes with immature cataracts. Hyper-mature lens cortices also had the high molecular weight aggregated proteins. In another study, enucleated human eyes were perfused with either lens homogenates or soluble proteins from cataractous lenses, and in both instances outflow was significantly decreased. These findings indicate that leakage of soluble protein aggregates from hypermature cataracts may be a significant factor in producing phacolytic glaucoma.

The functional state of trabecular meshwork cells as mediators of aqueous outflow has been considered. Cultured meshwork cells were found to secrete the

connective tissue elements, collagen and hyaluonic acid, and the overall glycosaminoglycan profile of these cells differed significantly from those of corneal fibroblasts or endothelial cells (possible contaminating cells). Continuing studies will consider whether irregularities in production of these elements may obstruct drainage channels and contribute to a decrease in outflow.<sup>20</sup> The establishment of human trabecular cells in culture has been reported by another investigator who also found them capable of synthesizing glucosaminoglycans.<sup>21</sup> Further characterization of trabecular meshwork cells by electron microscopy showed the presence of actin filaments in endothelium and in lesser amount in endothelial cells of Schlemm's canal.<sup>22</sup>

### Relationship of Glucocorticoid Sensitivity to Primary Open Angle Glaucoma

The observations that prolonged administration of glucocorticoid (GC) produces glaucoma-like symptoms and that patients with glaucoma tend to respond to those steroids with greater than normal increases in IOP have led to a search for the cellular basis of GC sensitivity. Several groups of investigators have used cultured fibroblasts or circulating lymphocytes as models to search for differences in cellular GC receptors between normal individuals and POAG patients. Correlations have shown glucocorticoid-induced increases of IOP in glaucoma patients with a two-fold increased sensitivity of their lymphocytes to GC (measured by inhibition of transformation). Recent findings are that POAG patients and matched normal controls had similar numbers of lymphocyte GC receptors and similar affinities of the cellular receptors for GC.<sup>23,24</sup> However, using prednisone, another type of GC, a significant difference in lymphocyte sensitivity was observed between cells from normal and POAG patients.<sup>25</sup> Glucocorticoid receptors of rabbit iris-ciliary body tissues were also found to have a high affinity and low capacity for GC similar to those seen in other tissues.<sup>26</sup> Autoradiography showed that the tritiated GC was located only in the ciliary epithelium.<sup>24</sup>

Glucocorticoids bind to nuclei of cultured fibroblasts from normal persons and POAG patients with the same order of affinity as they do to lymphocytes, and no significant differences in numbers of receptors were found between the two groups.<sup>23-27</sup> A possible control mechanism for fibroblast receptor sensitivity to GC is suggested by the observation that the nutritional status of the cells determines the direction of their response.<sup>27</sup> These various studies indicate that neither cytoplasmic nor nuclear binding of GC in the studied cells correlates with POAG, and that cellular sensitivity to GC probably is at some point secondary to the cellular steroid receptors.<sup>27</sup> Since the metabolic activity of trabecular meshwork cells may influence aqueous drainage and may be under GC control, it is of interest that in cultured cells GC is transported to the nucleus by cytoplasmic receptors where it binds with high affinity.<sup>28</sup>

### Prostaglandins

The possible roles of prostaglandins in glaucoma are of considerable interest for several reasons: they interact in a complex way in modulating noradrenergic mechanisms, both by mediating norepinephrine (NE) release and in being produced in response to NE, and they are also involved in inflammatory responses and have been used to produce a model for uveitis.

In a biphasic process in rabbits, topical norepinephrine causes an early rise in IOP, which is increased following superior cervical ganglionectomy, then a prolonged fall in IOP. Pretreatment with topical indomethacin (IND) prevents the rise in IOP, indicating that prostaglandins (PGs) are involved in the NE-mediated response. The later, prolonged NE effect, causing a drop in IOP, is not sensitive to indomethacin, indicating that it is a direct response to NE.<sup>29</sup> Further, IND treatment did not affect the adrenergic mediated changes in pupil size; mydriasis, therefore, is an NE effect. Measurements of jugular vein blood in control and bilaterally ganglionectomized animals showed that levels of cyclic AMP were significantly increased and those of  $\text{PGF}_{2a}$  significantly decreased in the laboratory animals.<sup>29</sup> Outflow facility in bilaterally ganglionectomized rabbits is increased by PGs and partially prevented by IND; this was previously thought to be a direct effect of NE.<sup>30</sup> In other experiments, the presence of thromboxanes (TBXs) and prostacyclins (PCYs) and their degradation products in ocular tissues was used as an index of NE-induced responses. It appears that PCY concentrations increase in iris sphincter and trabecular meshwork (stimulating cyclic adenylic acid production and smooth muscle relaxation) while  $\text{TBXA}_2$  affects ciliary process tissue (stimulating cyclic guanylic acid production and smooth muscle contraction). In vitro testing of tissues for NE-induced responses in the presence of specific inhibitors of PG synthesis confirmed the relative concentrations of PCYs and TBXs and their locations in iris ciliary body.<sup>31</sup>

Systemic administration of imidazole was found to inhibit the PGE-induced rises in IOP and in protein concentration of aqueous humor. Intraventricular  $\text{PGE}_1$  caused increases in IOP and body temperature which were blocked by aspirin. Either topical or systemic IND, which inhibits synthesis of PGs, reduced the increase in IOP which followed the intravitreal injections of arachidonic acid, the precursor of the prostaglandins, showing that they are formed intraocularly.<sup>32</sup>

The role of prostaglandins is relevant to the problem of glaucoma secondary to uveitis, because intraocular injection of PGs produces an inflammatory response accompanied by increased IOP. Topical and injected  $\text{PGE}_1$  and  $\text{PGE}_2$  potentiated conjunctival edema caused by histamine, while  $\text{PGF}_{2a}$  did not, suggesting that anti-PG drugs would be useful in treating external inflammations of the eye.<sup>33</sup> Ocular tissues were tested for the presence of enzymes synthesizing TBXs and PCYs. Microsomes prepared from human iris-ciliary body produced  $\text{TBXA}_2$ ; treatment with indomethacin abolished production of  $\text{TBXA}_2$ , but prostacyclin was still produced. In contrast, no  $\text{TBXA}_2$  was made by bovine iris, and this tissue produced a considerable amount of PCY and PGs.<sup>34</sup>

#### Drugs Used in Medical Treatment of Glaucoma

Carbonic anhydrase inhibitors have a valuable role in reducing aqueous humor formation in the treatment of glaucoma, but their use is limited by the severity of unacceptable side effects. In a recent study nearly half of the patients treated with acetazolamide had side effects characterized as "malaise syndrome," and the affected patients had a significantly higher incidence of acidosis than those not affected. Administration of sodium bicarbonate to the patients who were most acidotic alleviated their symptoms while not significantly altering the  $\text{CO}_2$  combining power of their sera.<sup>35</sup> If this observation is confirmed, the effective use of carbonic anhydrase inhibitors may be extended.

Adrenergic drugs continue to occupy a major place in the armamentarium of antiglaucoma drugs. Studies with specific  $B_1$  and  $B_2$  receptor agonists and antagonists have more fully defined the actions of these drugs. Both antagonists of  $B_2$  receptors, such as timolol, and  $B_2$  agonists lower IOP and outflow resistance, a seemingly paradoxical situation best explained by differing mechanisms and sites of actions. The reduction in IOP caused by timolol is primarily due to a decreased rate of aqueous formation by both peripheral and control mechanisms (as shown by dependence upon intact adrenergic innervation).<sup>36</sup> Timolol has been studied extensively for the past two years as trials of its efficacy and safety have progressed toward its approval by the Food and Drug Administration. Many short-term studies may be summarized as follows: timolol appears to be remarkably free of short-term ocular and systemic side effects; it significantly lowers IOP; it does not affect pupil size; it has good patient acceptance; it is generally better tolerated than epinephrine and is effective in a higher percentage of eyes; added to the drugs presently used in maximal therapy, it can further reduce IOP in certain patients. Thus far, no major deleterious effects have been seen on long-term use, although recent reports of tachyphylaxis have surfaced.<sup>37,38</sup> A long-term clinical trial is expected to begin shortly.<sup>39</sup>

An interesting experiment in the socioeconomic aspects of drug therapy, which also highlighted a dangerous error in drug dispensation, has been reported. Prescriptions were used to obtain brand or generically named antiglaucoma drugs in St. Louis and New York City; ranges in costs of drugs did not vary significantly in either city nor did they vary between rural and urban areas. However, drug substitution occurred in three instances when acetohexamide, an oral hypoglycemic drug, was substituted for the carbonic anhydrase inhibitor, acetazolamide. One patient was hospitalized for serious hypoglycemia-associated symptoms.<sup>40</sup>

Recently there has been increased interest in the potential of marihuana, and constituent or derivative cannabinoids, for treatment of glaucoma. The observation that smoking marihuana lowers intraocular pressure significantly has been confirmed in a number of studies. Possibly because of attitudinal biases, a variety of contradictory reports regarding the nature and incidence of side effects has appeared. It is generally agreed that conjunctival redness and a fall in systemic blood pressure are common consequences of smoking marihuana and that no notable ocular side effects occur. Basic physiologic and pharmacologic studies in animals indicate that the ocular effects of cannabinoids are mediated both by the central nervous system and by local ocular effects, in both instances by adrenergic mechanisms, and that both aqueous secretion (decreased) and facility of outflow (increased) are affected; furthermore, no tolerance was observed over a year's time.<sup>41,42</sup>

Limited anecdotal evidence has suggested that cannabinoids may be beneficial in treating glaucoma patients resistant to conventional medical therapy. Further controlled clinical research will be required to determine if, in man, cannabinoids will be of benefit to patients resistant to current drug therapy. If visual field loss will be limited, what physiologic and behavioral side effects may accompany chronic use, and if tolerance to clinical dosages develops with time. Optimal route of administration of controlled dosage will have to be established with respect to the above questions.

## Genetic Correlates of Glaucoma

Although glaucoma appears to have some genetic determinants, earlier reports of simple correlates in man of glaucoma incidence with genetic markers have not been confirmed. A number of investigators have determined that no simple relationships between HLA system antigens and incidence of POAG exist. That there may be as yet undetermined immunologic or biochemical correlates of POAG is suggested by examples of heritable glaucoma in animals.<sup>43</sup> A search for human or animal correlations with known enzyme polymorphisms might provide markers for inherited sensitivity of IOP to glucocorticoids or for predicting which ocular hypertensives will develop glaucoma.

## Animal Models

The best current nonprimate animal model for glaucoma is a strain of the beagle dog which has a chronic open-angle glaucoma condition. A colony of these animals has been established, and evidence indicates that the disease is transmitted as an autosomal recessive trait. Many ocular responses of these animals are similar to those of humans with POAG (although in the dog this condition ultimately progresses to a narrow-angle type of glaucoma) including biphasic responses in IOP to epinephrine, reduction of IOP with pilocarpine or acetazolamide, increased IOP following water-loading, progressive changes in disc appearance, and finally disc atrophy. Hindrance of aqueous outflow is indicated as the major cause of the disease.<sup>43</sup>

No heritable form of glaucoma has been reported in primates; therefore, studies with primates necessarily employ eyes in which pressure has been altered artificially by causing inflammatory reactions or by blocking outflow surgically (or by laser) or with particulates. Buphthalmic rabbits may have some value as models for glaucoma research, although they are not presently considered useful for POAG studies. Increased IOP, due mainly to impaired outflow facility, is one of several congenital anomalies in this animal. Studies with the rabbit are of intrinsic interest as an example of an inherited eye disease, and further work may define the biochemical and cellular bases for the decreased outflow. A continuing search for animal models with glaucomatous symptoms should be encouraged, with special attention to animals of genetically well-defined strains.

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## SENSORY AND MOTOR DISORDERS OF VISION

### Introduction

The recent report of the National Advisory Eye Council, Vision Research-A National Plan: 1978-1982<sup>1</sup>, defines the scope of this program as "...the structure and function of the neural pathways from the retina to the brain, the central processing of visual information, visual perception, optical properties of the eye, functioning of the pupil, and control of the ocular muscles." The anatomical structures involved are the eyeball as a whole, its accessory organs, and the parts of the nervous system and musculature involved in vision. Examples of sensory and motor disorders of vision are strabismus, amblyopia, nystagmus, degenerations of the optic nerve, abnormalities of gaze, third cranial nerve paralysis, and oculomotor dyslexia. The size of this public health problem is significant. Severe visual impairments resulting from more serious disorders such as strabismus, amblyopia, and optic nerve disease affect more than 3 percent of the population of the United States. When one considers the less severe impairment resulting from uncorrected refractive errors, it is no surprise to discover that over one-quarter of the visual problems in the United States are sensory or motor disorders.

Research on such a heterogeneous group of disorders requires the contribution of a large number of specialists: neuroanatomists, neurochemists, neurophysiologists, bioengineers, biomathematicians, psychophysicists, geneticists, and behaviorists, to mention the more well-represented disciplines working in this field. In order to provide a focus for the different types of research found in the program or to identify a group of diseases, six subprograms have been organized:

Congenital, Developmental, and Degenerative Abnormalities. This subprogram contains several genetic or developmental diseases. To understand them, as well as normal vision, considerable knowledge must be acquired concerning the genetics of normal development of the central visual system and of the eye itself. About one-fourth of the grants in the program is devoted to this subprogram.

Oculomotor Disorders. Some of the more serious disturbances are categorized here: strabismus, amblyopia, and nystagmus. In addition, considerable vision research aimed at understanding the cerebellar and brain stem organization of the oculomotor system is included. This subprogram is further divided into two areas, Strabismus and Oculomotor Control, and contains 15 percent of the active research grants in the program.

Optical and Pupillary Disorders. This subprogram contains research dealing with the most common visual disorder, refractive error and some less common but more serious diseases such as malignant myopia. Basic research on animal models in tracing neural paths for pupillary control are also included. This subprogram contains about 2 percent of the research grants in the program.

Visual Sensory and Perceptual Disorders. This subprogram is concerned with the processing of visual information at neural levels from the retinal

photoreceptors to the visual association areas of the cerebral cortex. It contains over 50 percent of the grants in the program and is further subdivided into Neural Mechanisms, Psychophysical Functions, and Electrophysiological Techniques Applicable to Man.

Sensory and Motor Disorders Related to Specific Disease Processes. Many diseases affect the central visual pathways but are not uniquely related to the visual system. When the relationship to the eye or the visual system is of primary importance, the investigation belongs in this division of the program. This subprogram currently contains about 1 percent of the grants and is subdivided into Vascular and Circulatory Abnormalities; Inflammatory Diseases; Metabolic, Toxic, and Traumatic Disorders; and Tumors.

Rehabilitation. This subprogram now contains less than 1 percent of the grants in the program, but it is likely to expand. In an area of such importance, of interest to so many agencies, fundamental scientific work will continue to be encouraged.

Here we will highlight the research accomplishments in only a few of the areas of greatest activity.

### Importance of Visual Experience

In recent years the early development of organisms has received increasing attention in several fields. Genetics and embryology are becoming important to research workers of all kinds. The field of vision research is no exception. Vision Research-A National Plan: 1978-1982<sup>2</sup> emphasizes the need for further knowledge concerning the development of the visual system. An active research area in the program deals with nervous system plasticity and critical periods in development.

Critical Periods. These occur throughout an organism's life. What is an insult at one developmental stage is neutral or even benign at another. Certain kinds of visual experience early in life are necessary for the proper development of the visual system of most mammals. It has been shown that monocular deprivation of vision can cause a dramatic shrinkage of the ocular dominance columns (series of cortical cells activated by light striking the retina) of the deprived eye and expansion of the nondeprived ones in the striate cortex of monkeys when the deprivation takes place during a critical period.<sup>3</sup> Several investigators agree that there is such a critical period in development in many parts of the visual system, but there is some disagreement as to when and how long this critical period takes place.

Some cortical cells are driven by binocular vision. Packwood and Gordon<sup>4</sup> found that the proportion of these cells, as well as performance on behavioral tests requiring stereopsis, was greatly decreased when kittens were raised in the dark except for one hour per day of monocular experience (alternating occlusion of the eyes) from birth to 12 weeks of age. Attempting to establish the age when a critical period occurs and its duration, Gordon and her associates<sup>5</sup> raised kittens in the light for five weeks, then alternately occluded one eye one hour per day while the rest of the kittens' time was spent in the dark. The monocular experience was varied from one to twenty-one days. Immediately

after this regime, the authors found that one or two days of alternating occlusion produced little effect but that ten or twenty days produced severe disruption of binocular driving in the cortex.

In an anatomical study, LeVay and his associates<sup>6</sup> found that afferents to the cortex were indistinguishable in kittens up to three weeks of age. The segregation into the columnar structure did not take place until kittens were three to seven weeks of age. Recording the receptive field properties of cells in the striate cortex of kittens aged nine to twenty-three days, Freeman<sup>7</sup> found that, in normally reared kittens, the vast majority of visually responsive neurons was binocularly driven. When one eyelid was sutured between nine days and fourteen days of age, cortical ocular dominance patterns shifted toward an input from the nondeprived eye.

Differences in estimates of the developmental stage of the critical period, its duration, the severity of the effect, and in other parameters can probably be accounted for by other variations in the way a study is designed. Daw<sup>8</sup> finds a difference in timing of the critical period during which insult produces changes in ocular dominance columns, on the one hand, or in directional-selectivity columns, on the other. It seems reasonable, then, that there should be a longer delay in development if the measures are behavioral. Heins<sup>9</sup> kittens had to negotiate a bridge and to walk through an obstacle course. Some kittens found this completely impossible--those that were reared in the dark to six weeks of age and were then operated upon. The III, IV, and VI cranial nerves were severed to immobilize the eyes, and vision was occluded in one eye.

In spite of some differences of opinions concerning when critical periods occur, there seems little doubt that they exist. One is left to speculate about the possibility of critical periods during development of vision during which usually traumatic events would have no or benign consequences.

Plasticity. This is a word with flexible meaning. In the present context one finds the word means: (1) no change resulting from an experimental manipulation where, one normally expects change; (2) permanent change resulting from an experimental manipulation where, a priori, one would not expect change; (3) change when an experimental manipulation is performed at one time but not when it is performed at another; (4) change in one anatomical area which produces no change in a related area; and (5) recovery from a serious disturbance. In one way or another, at one time in the life cycle or another, from one cause or another, plasticity of the visual system has been demonstrated in many species: cats, of course,<sup>10</sup> mice,<sup>11</sup> hamsters,<sup>12</sup> monkeys,<sup>13</sup> rats and dogs,<sup>14</sup> even toads,<sup>15</sup> and chickens.<sup>16</sup>

A common experimental technique used to study plasticity is monocular deprivation, produced by occlusion of one eye or lid suture. Drager<sup>11</sup> found that although the anatomy of the striate cortex of monocularly deprived mice showed little change, physiological testing demonstrated a decrease in the number of cells in the contralateral cortex activated by the deprived eye and a proportional increase in cells activated by the experienced eye. On the other hand, when LeVay<sup>17</sup> deprived adult cats and monkeys, there was no resulting increase

in the size of the ocular dominance columns representing the good eye and no evidence of shrinkage of the columns representing the other. Removal of one eye early in development causes the other to send a substantial ipsilateral projection to subcortical visual centers. Apparently, retinal cells which normally do not contribute uncrossed axons now become the source of ipsilaterally directed axons.<sup>18</sup>

Using toads, George<sup>15</sup> transplanted the eye rudiment of a haploid animal into a diploid host. The projections to the tectum were identical in extent for each eye. However, the volume of rod elements and the tectal receptive fields of ganglion cells were larger for completely triploid animals than normal. Squint induced early in the life of cats results in a more extensive callosal projection in area 17, spreading over 10 percent of the representation of the visual field rather than about 1 percent.<sup>14</sup> Although rearing hamsters in complete darkness has no apparent effect, rearing them in stroboscopic illumination decreases the directionally selective cells in the superior colliculus.<sup>12</sup> Certainly, lesions in areas 17 and 18 represent permanent change, at least in anatomy. Nonetheless, cats with such lesions have good spatial vision, including form discrimination, but demonstrate clear deficits in tests of acuity.<sup>10</sup> Apparently, these cortical areas serve at the threshold of functions but do not mediate them exclusively.

So, various experimental manipulations produce changes in the visual system and deficits in performance. Is there a plasticity that allows other structures to take over functions that have been disordered, are there other manipulations that overcome or avoid these consequences, are there periods in the life cycle when the deleterious results do not inevitably happen? The answer is a guarded "yes". The brain demonstrates another kind of plasticity. Chick embryo retinal cells, grown in vitro, then transplanted into newborn rat brains, continue to grow, divide, and even form some connections with host tissue.<sup>16</sup> Catecholamines seem important for the growth of visual connections in the cortex or for their preservation. Catecholamine-depleted kittens had a lower incidence of visual cortical neurons driven by the initially closed eye compared to kittens that were not depleted. The latter showed age-dependent recapture of cortical neurons after monocular lid suture. Some binocular cells can be preserved by injection of beta adrenergic receptor blockers.<sup>19</sup>

Drager enucleated the experienced eye of her previously monocularly deprived mice and tested cells in the binocular area ipsilateral to the remaining eye. After a long period of time, visual responses appeared among them, but the receptive fields were not as elaborated as normal.<sup>11</sup> Henderson's monkeys were raised with monocular deprivation--each eye at different times--and also had small retinal lesions. Even so, after delays of as long as 18 months, visual acuity improved greatly.<sup>13</sup> However, cats which were monocularly deprived for 20 days at various young ages had form discrimination and brightness discrimination deficits when each eye was tested separately. The deprived eye will gradually learn these tasks, but it never achieves the performance of the normal eye.<sup>20</sup>

Effects of age are very important. There is probably a time when the nervous system is extremely plastic, at least in part; there is undoubtedly a time

when at least part of the nervous system is not plastic at all. We have noted that LeVay's cats and monkeys, operated on in adulthood, showed no signs of anatomical change in the cortex after eye enucleation.<sup>17</sup> Behaviorally, prior binocular rearing had little effect if alternating occlusion was begun sometime in the critical period.

If one injects a label into a monkey fetus and allows the fetus to continue intrauterine development, one finds that neurons from both eyes have reached the cortex, and are beginning to segregate, by three weeks before birth. The segregation process is not completed until after birth. It is possible, then, that the changes that occur when an eye is sutured during the critical period could represent an arrest of normal development rather than an active rearrangement of neural connections.<sup>21</sup>

Binocularity. We have seen that there are all kinds of methods used by man and nature to achieve disruption of the visual system. Visual deprivation is one of these, and it seems there are innumerable methods used to achieve deprivation. From so many causes there are sure to be many effects. One of the most frequent concerns binocular vision. The way deprivation affects binocular vision is partly explained by an experiment conducted by Norton and his associates<sup>22</sup>. These investigators sutured the lid of one eye of tree shrews at about 7 days of age. Between four and twenty-four months of age the lateral geniculate nuclei were examined by single unit recording techniques. When cells were divided into X and Y categories, it was found that there was an almost complete absence of Y cells (presumably involved in peripheral stimuli and movement) in binocular portions of the nucleus. Y cells seemed relatively unaffected in the monocular portions; X cells were unaffected everywhere. The investigators found large numbers of abnormal cells in the binocular portion. These had some of the characteristics of Y cells but were not at all typical. It was assumed that these aberrant cells were replacements for the Y cells lost through the deprivation experience.

Pettigrew and Konishi,<sup>23</sup> studying the visual systems of several species of birds, found that the cortex of owls raised with one eyelid sutured showed severe deficit of cells that can be driven by either eye. The kestrel, a bird with laterally placed eyes, has a separate visual path linked to the central foveas. The owl lacks this pathway. The authors hypothesized that the eyes of a nocturnal animal, having a large aperture-to-focal-length ratio, would receive a very poor quality of image from objects in the periphery and, through evolution, would lose this pathway.<sup>24</sup> But nature permits some preservation of binocularity. Kittens reared with surgical rotation of both eyes do not have any remarkable disturbance of binocular cell distribution in the cortex.<sup>25</sup>

These are behavioral effects from these manipulations. Kittens which were raised with monocular deprivation did not show the expected deficits in negotiating a bridge or obstacle course if they received short binocular training before surgery for eye immobilization.<sup>9</sup> Kertesz has found that the eyes of normal humans will rotate about their axes, even in the opposite directions, in order to achieve a fusion of disparate images impinging on the two eyes.<sup>26</sup> There is a narrow strip of retinal ganglion cells straddling the nasal-temporal border which apparently decreases the fusion of the disparate images that the eye rotation is meant to achieve. When this area is masked, images of greater

disparity can be fused.<sup>27</sup> Richards<sup>28</sup> believes there are two kinds of stereopsis, one produced in the cortex and one subcortically. He has found evidence for this in both man and cats. Monocular cues in man, such as edges and contours, are important to the cortical mechanism of stereopsis. Without them, some humans are stereoblind.

### Eye Movement Control

Many of the recommendations for the Sensory and Motor Disorders of Vision program in Vision Research-A National Plan: 1978-1982<sup>29</sup> concern the workings of the eye muscles and their arrangement. One of the most active research fields in this program concerns eye movement control. We are beginning to understand parts of the entire system, but the way they interact is still far from explained. In this report we can only scratch the surface of some of the more interesting lines of research.

Signal Detection. Several investigators have identified two types of retinal ganglion cells but have designated them differently: X, Y; I, II; sustained, transient. Winters and his colleagues<sup>30</sup> feel that their data support the usual distinction between X and Y cells--analysis of form and movement respectively. They found that the maintained firing rate is higher for X cells than for Y. The temporal relationship between spikes from the center of the receptive field and from its surround are different for X and Y cells. The suppression of the center by stimuli in the surround is greater for X cells, and the resulting off-excitation for the center is greater for Y cells.<sup>31</sup> Although X and Y cells may be sensitive to location or movement of targets in the environment, further processing in the nervous system is necessary. Some visual neurons that signal retinal position do not activate eye movement, and other neurons signal particular saccades regardless of retinal location of the stimulus.<sup>32</sup> The further neural processing of information is complex. Retinal displacement of the stimulus is sometimes a sufficient condition for smooth eye pursuit movement, even without perceived motion.<sup>33</sup>

Cerebellum. One of the brain's relay stations for the control of eye movement is the cerebellum. One class of cells in the flocculus discharges at high levels during steady eye positions, stopping completely during a saccade. If a stimulus occurs to disrupt fixation, the flocculus releases its inhibitory action upon target neurons to allow a saccadic movement. Other cells in the flocculus are modulated in phase with target movement and eye velocity; it seems this area provides oculomotor centers with information of position, velocity, and acceleration necessary for pursuit movements.<sup>34</sup> In lobes VI and VII of the vermis there are cells which discharge only with saccadic eye movement and other cells that are completely suppressed during such movement.<sup>35</sup> Since discharge rate is a function of eye position, it would seem that the vermis relays information about the position of the eye in the orbit.

Cortex. Environmental information collected by the retina is, through way stations, delivered to the visual cortex in the occipital lobe. In this area, some cells are arranged in columnar structures. In some of these, one eye or another predominates. Hence, certain cells are more likely to be activated by stimuli impinging on one eye or the other; others are activated by both eyes. Other columns contain cells which seem to have a preferred orientation. That

is, objects vertical or horizontal to the line of gaze are more likely to be effective stimuli. There is no necessary relationship between the two sets of columns.<sup>36</sup> These preferences are plastic. At certain ages, manipulations such as raising animals with striped goggles can bias the cortex so that most cells prefer one orientation.<sup>37</sup> After alternating occlusion of one eye or the other of kittens and simultaneously alternating their environment between a vertically or horizontally striped drum, Gordon-Lickey<sup>38</sup> found groups of cells in which one eye was associated with one orientation and the other eye with the other orientation. However, this sharp preference could not be communicated to receptor cells in the superior colliculus where the usual broadly tuned directional selectivity remained.

Parts of the cerebral cortex other than the striate area are involved in visual processing, especially in its oculomotor aspects. The best known of these is area 8 of the frontal lobe, the frontal eye fields. These and other neurons discussed so far seem to discharge simultaneously with environmental events or with the movement of an eye muscle. The locus of the true switching mechanism, the point where a "decision" is made to move the eyes to a new point of fixation still eludes us. A candidate for such a locus of at least part of this switching mechanism is found in the inferior parietal lobe, area 7. A group of light sensitive cells there was described by Yin and Mountcastle.<sup>39</sup> Different cells represent different parts of the peripheral visual field. They are activated when a stimulus appears in the periphery of the visual field, before any eye movement starts and whether or not a saccade is made in that direction. They do not represent the fovea and stop discharging with a saccade in the appropriate direction.

Midbrain. This is another of the relay stations transmitting information to oculomotor muscles. The superior colliculus (SC), receiving information from several sources, does not transmit the same information directly to muscles. The pattern of single neuron firing is unrelated to saccade direction or amplitude; the information appropriate for muscle movement must be contained in the spatial distribution of neurons in the SC.<sup>40</sup> This spatially coded information is transmitted from the SC to the medial pontine reticular formation (MPRF). One group of cells in the MPRF transforms the code to a temporal one. This information then is transmitted to cells which seem to control motoneurons. Another group of cells in the MPRF receives information from the SC which inhibits the cells that control motoneurons and "gate" saccades precisely.<sup>41</sup> Experimental manipulations involving both eyes simultaneously can have plastic effects on directional selectivity of cells in the superior colliculus. For example, Chalupa and Rhoades<sup>42</sup> demonstrated a marked decrease in the incidence of directionally selective cells there as the result of raising hamsters in stroboscopic illumination. This treatment permits pattern vision but not the perception of movement.

Muscle Movement. Muscle physiology and coordination of eye muscles have been extensively studied. Yet, there remain some anomalies. Saccades are not always as precise, moving immediately to the peripheral stimulus and immediately stopping, as we have thought. In some persons, saccades are characterized by an overshoot of the target with an immediate reversal of gaze to halt the eye and bring it to the target. The overshoot seems greater for small saccades.<sup>42</sup> Intracellular stimulation of abducens motoneurons results in contraction of

two eye muscles which are not antagonistic but are functionally distinct. Stimulation of the oculomotor nucleus and of the trochlear nucleus both result in contraction of the ipsilateral lateral rectus muscle. Stimulation of the trochlear nucleus and of the abducens nucleus both result in contraction of the ipsilateral inferior oblique muscle.<sup>43</sup>

Perception. Regardless of the anomalies in the eye-movement system and our lack of understanding of them, perception is not only possible but essential for the complete operation of the visuomotor system. In fact, it takes all the experimenter's ingenuity to arrange conditions that distort the system. Eye movement information is constantly fed into this perceptual system, the contact with the outside world. Often, it is this kind of information which produces perceptual illusions such as moving objects which seem to stand still or move in the opposite direction if they appear in a frame which moves faster in the same direction.<sup>44</sup> Pasternak's<sup>45</sup> cats, reared in stroboscopic illumination, had difficulties in the placing response, in negotiating obstacle courses, and in grating orientation discrimination as late as twelve months of age. Over a long period of time, with intensive training efforts, the cats responses returned close to normal--another demonstration of plasticity in the central nervous system.

### Special Initiatives

As in prior years, the Sensory and Motor Disorders of Vision program of the National Eye Institute has undertaken special initiatives in a number of areas. During fiscal year 1978, particular effort has been expended in four areas: (1) low vision, (2) clinical application of psychophysical and physiological optics techniques, (3) improved fundamental and clinical utilization of eye-tracking technology, and (4) clinical trials. Activities under these headings, in contrast to other parts of this Annual Report, cannot be reported as scientific advances but rather as administrative accomplishments in identifying areas where more knowledge is needed, communicating these needs to the research community, and establishing the mechanisms for achieving resolution of the needs.

Low Vision--Rehabilitation. Many persons have sight which has been permanently and irreversibly impaired. Although not totally blind, many have loss of sight sufficient to interfere with gainful employment, limit independent activity, hamper communication, and in general restrict the range of activities that comprises daily living. An excellent definition of low vision has recently been presented by scientists at the Eye Research Institute in Boston:<sup>46</sup> Low vision is residual vision. It is "the vision one has left...after all the medical treatment of the cause of the disability has been utilized." Low vision can be the result of "any pathological, congenital, or traumatic condition of the eye which results in a decrease of central vision or peripheral field and which is not amenable to medical, surgical, or ordinary optical (management)."

In Vision Research--A National Plan: 1978-1982 the National Advisory Eye Council identified the need for additional research directed toward objectives in visual rehabilitation.<sup>47</sup> As a first step in this direction, a workshop on "Research Opportunities Relevant to the Management of Severe Visual Impairment" was conducted by the National Eye Institute in June 1977. The focus of this

workshop was on recent advanced and current needs in the areas of rehabilitation of the low-vision individual.

As a result of the Council recommendations and the activities of the workshop, a program announcement was developed. The program announcement<sup>48</sup> requested grant applications in the following (and related) research areas: (1) better diagnosis of visual-system impairments and improved characterization of residual vision, (2) development and evaluation of special devices and techniques to improve visual performance of patients with specific optical or retinal irregularities, (3) development and clinical trials of special devices and techniques to improve mobility and the performance of jobs and skills, and (4) assessment of visual functions in the infant and young child.

Application Of Psychophysical And Physiological Optics Techniques To Clinical Problems. Visual testing and assessment in the clinic depends, to some extent, upon the skill and experience of the examiner as well as the verbal responses of the patient. These subjective procedures are also influenced by physical, psychological, and environmental factors that confound the evaluation of the patients' condition. In the clinical research setting, these uncontrolled factors limit the investigator's ability to differentiate diagnostic groups and to evaluate the effects of treatment.

Scientists in psychophysics and physiological optics laboratories have developed techniques for controlling stimulus and measurement variability during testing. They have also developed new techniques for objective measurement of the various parameters of visual functional capability. With the intent of transferring these techniques and approaches from the fundamental science laboratory to clinical research and to patient evaluation, the National Eye Institute sponsored a workshop during fiscal year 1977 on "The Role of Psychophysics and Physiological Optics in Ophthalmic Diagnosis and Patient Evaluation." A major result of the workshop has been the development and promulgation of an NEI program announcement<sup>49</sup> reflecting the need to bring these techniques to bear on such problems as localization of anomalies in glaucoma, senile macular degeneration, amblyopia, degenerations of the optic pathway, cerebral lesions, strabismus, disorders of oculomotor control, etc. While major advances in this field have been reported in prior Annual Reports (for example at the University of Florida,<sup>50</sup> Mt. Sinai Medical School,<sup>51</sup> and the Massachusetts Eye and Ear Infirmary<sup>52</sup>), these are but at the forefront of a major new area of translation of research findings in which NEI staff have been intimately involved on a day to day basis with major eye research programs in the United States.

Improved Fundamental And Clinical Utilization Of Eye-Tracking Technology. In the early 1970's, scientists at Stanford Research Institute<sup>53</sup> announced the adaptation of an instrument originally conceived for the U. S. space program to problems of eye position monitoring and tracking. This instrument, the Purkinje-Image Eye-Tracker, proved to be useful in tracking the state of accommodation of the eye, in measuring the position of the eye, and in image stabilization. Its primary advantages over prior instrumentation were the capabilities for high tracking accuracy without eye contact.

Because of these advantages, some 20 instruments have been obtained and utilized for research purposes by investigators across the United States.

Recognizing the potential for the application of this technology to the diagnosis and treatment of ocular diseases, in addition to its utility in further defining normative processes, the NEI staff has been working closely with this group of investigators. As a result, a National Eye-Tracking Study Group was formed in 1977 to foster cooperative fundamental and clinical research and to identify areas for improvement of eye-tracking technology and its application. The National Eye Institute and the National Advisory Eye Council has supported the activities of the National Eye-Tracking Study Group and Stanford Research Institute in pursuing the following objectives: (1) to evaluate and compare the utility of various eye-tracking techniques in the assessment of visual function and as clinical tools; (2) to recommend and pursue further development and application; and (3) to promote the exchange of information.

While a number of areas for potential application of this technology have been identified, the NEI staff has been working closely with the research community, and other agencies such as NASA and RSA, in planning for changes in instrumentation design and for conduct of initial studies in the following areas: (1) psychophysical testing of visual function, (2) electrophysiological recording from highly localized retinal regions, (3) high magnification funduscopy, (4) retinal photocoagulation close to the fovea, (5) two-point fluorometry, (6) proton-beam retinal cancer therapy, and (7) with a binocular eye-tracker system, measurement of fixational behavior in patients with extra-ocular-muscle or neurophthalmological disorders.

Clinical Trials. Myopia is a condition that affects a very large part of our population. While the etiology of nearsightedness is far from clear, "treatment" in the form of corrective lenses is traditional. In recent years it has been noted by Miller,<sup>54</sup> Rengstorff,<sup>55</sup> and many others that corneal curvature may be changed by contact lens wear and that consequently the refractive error in vision may also be altered. Since such changes may persist, some practitioners have attempted to exploit the changes in corneal curvature induced by shaped contact lenses to reduce refractive error permanently, a procedure known as "orthokeratology."<sup>56</sup> Orthokeratology, however, has remained unvalidated and controversial. Therefore, investigators at the University of California, Berkeley, have initiated recently a controlled clinical trial to compare the efficacy and safety of this therapy with traditional contact lens therapy.

The Sensory and Motor Disorders of Vision and Rehabilitation program is also supporting initial activities with respect to two additional prospective, randomized, controlled, clinical trials for which manuals of procedure are currently being developed. One is a proposed evaluation of the efficacy of over-correction in slowing the rate of progression of myopia in children.<sup>57</sup> The second is a proposed evaluation of a pharmacological alternative to eye-muscle surgery in strabismus.<sup>58</sup> Following completion of initial studies and manuals of procedure--and before initiation of the trial--the plans for each study will be evaluated by NEI staff and external consultants for soundness of experimental design, inclusion-exclusion criteria, measurements and procedures, attention to side effects, safeguards, and plans for informed consent.

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OFFICE OF BIOMETRY AND EPIDEMIOLOGY



ANNUAL REPORT  
NATIONAL EYE INSTITUTE  
October 1, 1977 - September 30, 1978

REPORT OF THE CHIEF, OFFICE OF BIOMETRY AND EPIDEMIOLOGY  
Fred Ederer

A large part of the Office's activities has been conducted by the Clinical Trials and Natural History Studies Section, which provides scientific staff support to three large multicenter trials in diabetic retinopathy supported by the National Eye Institute. The oldest of these, the Diabetic Retinopathy Study (DRS), has entered its last year of data collection, which will be followed by two years of wind-up and report writing, and the youngest, the Early Treatment of Diabetic Retinopathy Study (ETDRS), is nearing the completion of the preparation of a manual of operations. This Section has also collaborated in research activities related to the intraocular lens, the role of nerve bundle fiber defects in the diagnosis of glaucoma, and the treatment of uveal melanomas.

Because of an acute shortage of epidemiologists, the Office of Biometry and Epidemiology has, in the past, been unsuccessful in attracting qualified candidates for the position of Head, Epidemiology Section. The lack of senior epidemiology staff has impeded the development of an epidemiology program. A search committee of national prominent epidemiologists has now been appointed to assist in the recruitment. The committee is expected to complete its work before the end of 1978.

New initiatives were undertaken during the past year in exploring the feasibility of studies of incidence and prevalence of eye disease and visual impairment, but the implementation of such studies will have to await the recruitment of staff. In the meantime, work was continued on a statistical monograph and other analyses of the Framingham Eye Study and on the India cataract study. The Office succeeded in obtaining statistical data tapes from the ophthalmic and medical examinations of the 1971-72 Health and Nutrition Examination Survey (HANES), and is planning to prepare some special epidemiologic analyses for publication from these data.

For the second year in a row, the Office conducted a course in methods of clinical research at the annual meeting of the American Academy of Ophthalmology, held in Dallas. Also, a contract proposal, now under review, has been solicited from Dartmouth University for conducting a two-day course in methods of clinical research for ophthalmologists in three different locations in the United States.



## BIOMETRY SECTION

The Biometry Section continues to assist and collaborate with investigators, both within and outside NEI, in applied statistical and epidemiologic studies.

Mr. Marvin Podgor is collaborating with Dr. Robert Frank, Kresge Eye Institute, Wayne State University, in a study of retinal vascular changes in juvenile onset diabetes of short duration. He also assisted the NEI Laboratory of Vision Research with bioassay analyses. Dr. Cristina Leske, State University of New York at Stony Brook, presented a paper with Mr. Podgor on estimation of glaucoma incidence from prevalence data at the annual meeting of the Society for Epidemiological Research.

Dr. Roy Milton consulted with Dr. David Sommer and Dr. Jonathan Pederson of the NEI Clinical Branch on analyses of axial length of sutured monkey eyes by ultrasonography and of effects of experimental choroidal detachment on intraocular pressure and aqueous flow, and he continued his consultation with Dr. Douglas Gaasterland on studies of parameters of aqueous humor dynamics. Dr. Milton provided extensive assistance to Dr. Jack Hahn, NIH Division of Research Resources, in the establishment of a contract for the BMDP biomedical computer programs, formerly supported by a research grant. Together with Dr. Arin Chatterjee, Christian Medical College, Ludhiana, India, Dr. Milton is completing a cataract etiology study of three areas in the Punjab.

The Framingham Eye Study contract is monitored by Dr. Milton as Project Officer and other members of the Biometry Section. A final file of the ophthalmic data from this study will be documented and made available for general research use by the end of the year. Mrs. Helen Moorhead and Dr. Milton are providing close assistance to this study, especially in the production of a detailed statistical monograph as the study approaches completion in December.

Invited talks on the Framingham Eye Study were presented by Dr. Milton at the meeting of the International Epidemiological Association in Puerto Rico and at the national conference of the National Society for the Prevention of Blindness in New York. He also presented an invited paper in May at the U.S.-Japan Conference on Biostatistics in the Study of Human Cancer, Hiroshima, Japan, sponsored by the National Cancer Institute.

Mr. Podgor, with Mr. Ederer, completed Biometrics Note 6, "Estimates of a hypothetical delayed deleterious effect of photocoagulation treatment for diabetic retinopathy," and a subsequent update, based on the Diabetic Retinopathy Study. Dr. Milton was author or coauthor of three papers appearing this year; one additional paper is in press.



## EPIDEMIOLOGY SECTION

The primary function of the Epidemiology Section is to develop and conduct a program of epidemiological research in eye disease, with special emphasis on the chronic diseases that cause blindness and visual impairment in the United States. The epidemiologic investigations are intended to uncover clues about etiology and pathogenesis and include prevalence surveys, case-control studies, population genetic studies, and studies directed at developing and improving diagnostic methods suitable for epidemiologic research.

Primary epidemiologic indices are incidence and prevalence of disease in defined populations. Little such information exists for eye disease. Geographic differences and time trends in disease frequency often suggest hypotheses for further research or offer evidence to support or contradict existing hypotheses. Initial steps were taken toward the development of such data. First, an outline of a plan was prepared by Mr. Harold Kahn for a three-stage national survey of visual impairment and its causes. The feasibility of the plan is now being discussed with representatives of the HEW National Center for Health Statistics. Second, the feasibility of modifying existing national surveys of medical care providers to enable the collection of eye disease incidence information was explored by Mr. Theodore Woolsey, a private consultant. A report is in preparation. Third, the National Eye Institute is cooperating with the National Institute of Neurological and Communicative Disorders and Stroke in a study exploring the feasibility of modifying the extant National Hospital Discharge Survey of the National Center for Health Statistics to allow for the collection of incidence data for such diseases as cataract.

The Office of Biometry and Epidemiology has received copies of several of the data tapes, including those of the ophthalmic and medical examinations, of the 1971-72 Health and Nutrition Examination Survey (HANES) conducted by the National Center for Health Statistics. The survey included eye examinations of some 10,000 individuals from a probability sample of the United States population. The Office plans to use these data for special epidemiologic studies.

A small contract was issued to the University of Arizona to assemble data related to the question of whether the prevalence of cataract is related to exposure to sunshine. The data will be processed and analyzed at the National Eye Institute.



## CLINICAL TRIALS AND NATURAL HISTORY STUDIES SECTION

The principal activities of this Section are conducting randomized clinical trials on the prevention and treatment of eye disorders, performing non-randomized trials on their natural history, and providing consultation to colleagues in other parts of the NEI who are also active in clinical trials research. Of these, the scientific management of clinical trials was the dominant activity during FY 1978. Responsibility for this activity is shared by project teams assigned to each study, led by a staff member of OBE, and including NEI personnel with expertise in biostatistics, ophthalmology, computer science, contract management, and public information.

Two papers have been published on the Diabetic Retinopathy Study (DRS), our multicenter clinical trial of the efficacy of photocoagulation in the treatment of diabetic retinopathy. They report that photocoagulation is effective in reducing the risk of severe visual loss from diabetic retinopathy and in inhibiting the progression of the disease. These effects were apparent in all stages of diabetic retinopathy studied: proliferative, severe nonproliferative, and background. Some deleterious effects of treatment were also found, namely, small losses of visual acuity and constriction of the peripheral visual field. Follow-up of all surviving DRS patients will continue until May 31, 1979. Data analyses for a series of papers on the following topics are in preparation: detailed description of DRS methods and baseline characteristics of patients and eyes, detailed description of treatment effects, macular edema, and assessment of risk factors of severe visual loss and death.

The Early Treatment of Diabetic Retinopathy Study (ETDRS) is a new multicenter randomized trial begun during FY 1978 and designed to answer some of the major questions not addressed by the DRS and other studies. The important issues are identification of the earliest stage of disease in which photocoagulation should be initiated, the extent to which the laser treatment should be scattered, and the value of aspirin and/or dipyridamole in slowing progression of the retinopathy. A first draft of a Manual of Operations for the study is expected to be completed by fall of 1978, and patient recruitment is expected to begin in the spring of 1979.

The Diabetic Retinopathy Vitrectomy Study (DRVS) is a randomized clinical trial involving treatment of a relatively advanced stage of diabetic retinopathy in which blindness due to hemorrhage into the vitreous has occurred. The surgical procedure being examined is vitrectomy, using an instrument combining cutting, suction, and infusion of a replacement solution. Eligible eyes are assigned to either vitrectomy within the first six months of a vitreous hemorrhage or to a "late" group in which vitrectomy is performed one year following hemorrhage in those eyes still suitable for treatment. Over 200 eyes were randomized by fall of 1978, and several patients were already eligible for deferred surgery. During FY 1979 the important evaluation of 18 month data following hemorrhage will become available.

With improvements in the design of plastic lenses for intraocular implantation following cataract removal, the number of such implants has been increasing rapidly. Many surgeons have felt that it is important to evaluate the efficacy of such implants in patients undergoing cataract extraction and

to assess the risks as well. As a first step toward evaluating the safety of intraocular lens implants, the National Eye Institute has awarded a contract to examine the feasibility of using the clinical records of several ophthalmologists with large numbers of patients in whom such lenses have been implanted. If these records, though designed for patient care, are also suitable for research purposes, analyses of complication rates will follow. By the end of 1978, the feasibility of such retrospective studies will be known.

The Section has accepted responsibility for consulting on clinical trial protocols developed under NEI grants. This has turned out to be a useful administrative mechanism by which the Institute helps to assure the quality of grant-sponsored clinical trials. It offers the additional benefit of promoting communication between the Section staff and NEI Extramural Program staff on activities of shared interest.

Considerable interest has been shown by ophthalmologists in recent months in evaluating alternative treatments for uveal melanoma. The Institute supported a workshop in July 1978 to explore the feasibility of a number of research designs with the aim of developing information that would be helpful to potential applicants for grant funds. Dr. Seigel assisted in planning the meeting and represented the Institute as a statistical consultant.

The Section has also been involved in cooperative arrangements involving other governmental components. Dr. Lawrence Rand has served on an advisory group to the National Institute on Arthritis, Metabolism and Digestive Diseases to determine the feasibility of developing a clinical trial on the effect of metabolic control on the development of the vascular complications of diabetes. Dr. Seigel represents the NEI at the NIH Committee on Clinical Trials.

## CONTRACT NARRATIVE

Nineteen Clinical Centers, plus a Coordinating Center at the University of Maryland School of Medicine, Baltimore, Maryland; a Reading Center at the University of Wisconsin, Department of Ophthalmology, Madison, Wisconsin; and a Central Laboratory at the Center for Disease Control, Atlanta, Georgia

Title: Early Treatment of Diabetic Retinopathy Study (ETDRS)

Principal Investigator: Dr. Lloyd Aiello

Current Fund Allocation: \$1,272,608 for the period September 9, 1977, through September 30, 1978.

Objectives: The Early Treatment of Diabetic Retinopathy Study (ETDRS) is a multicenter, randomized clinical trial, the main goals of which are:

- a. To determine whether treatment of early stages of proliferative and nonproliferative diabetic retinopathy with or without macular edema by aspirin and/or dipyridamole and/or prompt photocoagulation is effective in decreasing the rate of development of known retinopathy risk factors and/or the development of severe visual loss when compared to placebo or deferred photocoagulation.
- b. To determine the optimum time to initiate photocoagulation treatment in diabetic retinopathy.
- c. To monitor closely the effects of diabetes mellitus and/or of photocoagulation on visual function.
- d. To develop natural history data that can be used to develop or confirm etiologic hypotheses or identify risk factors in diabetic retinopathy.

Major Findings: The first general session of ETDRS Investigators took place in Bethesda on November 29-30, 1977. One of the main objectives of that meeting was to begin the planning phase of the study. The Protocol and Procedures Committee as well as other committees started their activities at this session. Subsequent meetings were held on January 16, 1978, in Seattle; on February 22 and on March 18 at O'Hare Airport in Chicago; on April 30 in Sarasota, Florida; and on June 5, 1978 in Philadelphia.

The completion of the Study design and preparation of the Manual of Operations are scheduled for November 1978. Recruitment of patients is expected to begin by Spring 1979.

Significance to Biomedical Research and the Program of the Institute: The Institute regards careful evaluation of widely used treatments as an essential element in its program of research. There is a need to conduct basic research that has potential to develop procedures that prevent or treat ophthalmologic disorders. There follows a need to evaluate such procedures

to assure the public and the medical community of their high quality. This study represents an extension of the Institute's interest in developing the best possible program to care for the patient with diabetes.

Proposed Course: Follow-up of all ETDRS patients will continue for five to eight years, and monitoring of accumulating data will be performed at three-month intervals.

NEI Research Program: Retinal and Choroidal Diseases--Diabetic Retinopathy and Other Vascular and Circulatory Abnormalities.

Publications: None

## CONTRACT NARRATIVE

Thirteen Clinical Centers, plus a Coordinating Center at the University of Maryland School of Medicine, Baltimore, Maryland and a Reading Center at the University of Wisconsin, Department of Ophthalmology, Madison, Wisconsin

Title: Diabetic Retinopathy Vitrectomy Study (DRVS)

Principal Investigator: Matthew D. Davis, M.D.

Current Fund Allocation: \$1,254,000 for the period June 25, 1978, through June 26, 1979.

Objectives: The DRVS is a multicenter clinical trial to:

- a. Evaluate vitrectomy performed in the first six months after vitreous hemorrhage secondary to diabetic retinopathy, as compared to the more usual practice of waiting twelve months after vitreous hemorrhage to remove the vitreous.
- b. Collect natural history data on patients who have diabetic retinopathy with extensive formation of abnormal blood vessels and/or early retinal detachment, but without extensive vitreous hemorrhage.

Major Findings: As of May 31, 1978, a total of 780 eyes had been found eligible for the second baseline visit: 232 are eligible for vitrectomy and 548 are eligible for the natural history component of the trial. 184 eyes have been randomized to either early or late vitrectomy and 502 eyes are enrolled for natural history. The 232 eligible eyes represent 28% of the vitrectomy recruitment goal of the DRVS.

Significance to Biomedical Research and the Program of the Institute: Diabetic retinopathy is one of four major causes of adult blindness and differs from the other three (macular degeneration, glaucoma, cataract) in that it affects a younger population. A major cause of this blindness is vitreous hemorrhage. Vitrectomy has been shown to be of some benefit to individuals who have had a severe vitreous hemorrhage for at least one year, and it is thought that diabetic blindness can be further reduced if vitrectomy is performed at an earlier date. This presents an ideal opportunity for the National Eye Institute to organize scientific talents to answer a significant medical question.

Proposed Course: All 13 clinics are actively recruiting and enrolling patients in the study. The Coordinating Center is processing all the data forms, providing interim results for review by the Data Monitoring Committee, and monitoring the recruitment efforts by each of the clinics. The Reading Center continues to grade the baseline fundus photographs and is now also processing the posttreatment photographs for both the early and deferred vitrectomy groups.

NEI Research Program: Retinal and Choroidal Diseases--Diabetic Retinopathy and Other Vascular and Circulatory Abnormalities/Vitreous Humor

Publications: None

## CONTRACT NARRATIVE

J. Robb Associates (NIH 1 EY 8-2100)

Title: Safety and Efficacy of Artificial Lens Implants

Principal Investigator: Jay Glasser, Ph.D.

Current Fund Allocation: \$59,522 for the period November 28, 1977, through November 27, 1978.

Objectives: Intraocular lens implants are being inserted by ophthalmologists into eyes following cataract surgery with increasing frequency. There is a need to evaluate the safety of these lenses. As a first step towards such an evaluation, the contractor will examine the cataract patient records of ophthalmologists who have implanted a large number of intraocular lenses. He will try to determine whether such records contain sufficient research quality information on relevant variables prior to, during, and after surgery, and whether long-term follow-up information on nearly all patients is available. These criteria will determine the feasibility of research on intraocular lenses using physicians records that were originally designed for patient care.

If the feasibility of such research is assured, then the contract will enter into an analysis phase, when data concerning the efficacy and complications of intraocular lens implants will be accumulated, analyzed, and published.

Major Findings: None

Significance to Biomedical Research and the Program of the Institute: This contract is important in two ways. First, it will help us learn more about the feasibility of research using physician records designed primarily for patient care. Second, it may yield important information on the safety of a rapidly growing ophthalmologic practice.

Proposed Course: By end of summer 1978, five visits will have taken place to ophthalmologists' offices. Three more will be scheduled. In the fall, analyses will be conducted of quality of records in these offices, and a decision will be made as to whether a retrospective scientific evaluation of their records is feasible.

NEI Research Program: Cataract--Senile or Degenerative Cataract

Publications: None



## CONTRACT NARRATIVE

Fifteen Clinical Centers plus a Coordinating Center at the University of Maryland School of Medicine, Baltimore, Maryland, and a Reading Center at the University of Wisconsin, Department of Ophthalmology, Madison, Wisconsin

Title: Diabetic Retinopathy Study (DRS)

Principal Investigator: Matthew D. Davis, M.D., Chairman

Current Fund Allocation: \$2,125,569 for the period June 30, 1978 through June 29, 1979.

Objectives: The Diabetic Retinopathy Study (DRS) is a multicenter clinical trial to evaluate the efficacy of photocoagulation (argon laser and xenon arc) in the treatment of proliferative diabetic retinopathy. This randomized, controlled study involves over 1,700 patients enrolled at 15 medical centers.

Major Findings: Photocoagulation with either argon laser or xenon arc, as used in the study, is effective in reducing the risk of severe visual loss and in inhibiting the progression of retinopathy. These effects were apparent in all stages of diabetic retinopathy studied: proliferative, severe nonproliferative, and background. Also found were some deleterious effects of treatment, namely, small losses of visual acuity and constriction of the peripheral visual field.

Significance to Biomedical Research and the Program of the Institute: Diabetic retinopathy, uncommon only a few decades ago, is now a leading cause of blindness and visual disability in the United States. There is a critical need to find and scientifically evaluate treatments which will reduce the risk of blindness or visual impairment from the ocular complications of diabetes. Although photocoagulation is widely used as a treatment, adequate evidence of its efficacy is not based on carefully documented research findings.

Proposed Course: Follow-up of all surviving DRS patients will continue until May 31, 1979. This will be followed by a one to two year period of data editing, processing, analysis, and report writing. Data analyses for a series of papers on the following topics are in preparation: detailed description of study methods and baseline characteristics of patients and eyes, detailed description of treatment effects, macular edema, and assessment of risk factors of severe visual loss and death.

NEI Research Program: Retinal and Choroidal Diseases--Diabetic Retinopathy and Other Vascular and Circulatory Abnormalities

Publications:

The Diabetic Study Research Group: Photocoagulation treatment of proliferative diabetic retinopathy: The second report of Diabetic Retinopathy Study findings. Ophthalmology 85:82-105, 1978.



## CONTRACT NARRATIVE

Boston University (NIH-NEI-2112)

Title: Framingham Eye Study

Principal Investigator: Howard Leibowitz, M.D.

Current Fund Allocation: \$752,579 for the period July 1, 1972, through December 31, 1978.

Objectives: The aim of this epidemiologic investigation is to identify individuals among the Framingham Heart Study cohort who at the present time have a disease or condition related to one or more of the four most common causes of adult blindness, i.e. senile cataract, senile macular degeneration, chronic simple glaucoma, and diabetic retinopathy. In addition to determining the prevalence of these diseases, past measurements from the Framingham Heart Study will be related to present disease status in an effort to identify risk factors.

Major Findings: As previously reported, an ocular examination according to a standard protocol was made on the survivors of the original Framingham Heart Study cohort. Patient examinations were completed in February 1975 with 2,675 individuals examined. This includes 84% of the cohort still residing in the Framingham area. The Study's first two major reports included findings of significant association between senile cataract and increases in serum phospholipids, casual blood sugar, blood pressure, and age. Senile macular degeneration was found to be associated with increased blood pressure, ventricular hypertrophy, history of lung infection, aging-related factors, and sex. Prevalence in this population was 3% for open-angle glaucoma, 3% for diabetic retinopathy, 9% for senile macular degeneration, and 15% for cataract. A final data file has been delivered to the NEI for use in ongoing analysis by NEI and other investigators, providing a valuable data resource for future research. A statistical monograph is being prepared under sub-contract to the Biostatistics Center, George Washington University.

Significance to Biomedical Research and the Program of the Institute: The four eye diseases under consideration are the leading causes of adult blindness in this country today. It will be very helpful to identify factors possibly associated with increased risk of these diseases as a guide to prevention. The Study has been designed with this objective in mind. Prevalence data for this age group (52-85) in this community will be a useful by-product.

A comprehensive statistical monograph, now being prepared, will be a reference to the Study's detailed methodology and to the ophthalmic examination data. This publication will provide valuable information and guidance to investigators pursuing similar studies in the future and, through wide dissemination of detailed study data, will be a potential resource for answering future questions and stimulating additional research.

Proposed Course: Submission of the monograph to the Archives of Ophthalmology is expected in October 1978, and the contract will be completed on schedule. Further data analyses and research papers are being planned by staff members of the Office of Biometry and Epidemiology.

NEI Research Program: Retinal and Choroidal Diseases--Macular Diseases/Diabetic Retinopathy and Other Vascular and Circulatory Abnormalities; Cataract--Senile Cataract; Glaucoma--Primary Open-Angle Glaucoma.

Publications:

Milton RC: The Framington Eye Study. Sight-Sav Rev 48:29-36, 1978.

Office of Biometry and Epidemiology

The Diabetic Retinopathy Study Research Group: Photocoagulation treatment of proliferative diabetic retinopathy: The second report of Diabetic Retinopathy Study findings. Ophthalmology 85:82-106, 1978.

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Coller BS, Frank RN, Milton RC, Gralnick HR: Plasma cofactors of platelet function: Correlation with diabetic retinopathy and hemoglobin A(Ia-c). Ann Intern Med 88:311-316, 1978.

Milton RC: The Framingham Eye Study. Sight Sav Rev 48:29-36, 1978.

Gaasterland D, Kupfer C, Milton R, Ross K, McCain L, MacLellan H: Studies of aqueous humour dynamics in man: VI. Effect of age upon parameters of intra-ocular pressure in normal human eyes. Exp Eye Res 26:651-656, 1978.



OFFICE OF PROGRAM PLANNING AND SCIENTIFIC REPORTING



ANNUAL REPORT  
NATIONAL EYE INSTITUTE  
October 1, 1977 - September 30, 1977

REPORT OF THE CHIEF, OFFICE OF PROGRAM PLANNING AND SCIENTIFIC REPORTING  
Julian M. Morris

Program Planning

The Office's primary activity in program planning during the year was assisting the National Advisory Eye Council in completing its second major planning report, Vision Research---A National Plan: 1978-1982. This report, published in April, identifies major research needs and opportunities in vision research based on diverse analyses of the state of the art of vision research by over 160 expert consultants. In preparing this report for publication, the Office coordinated and edited all contributions by the Council and its consultants from the vision research community, NEI staff, and NEI contractors. The Office staff developed the report's format, drafted much of the introductory and summary material, developed over 35 pages of resource tables which presented the Council's priorities in terms of recommended projects, developed the other charts and tables used in the report, obtained photographs or had them taken, and prepared the index. The Office staff also supervised the report's graphic design and layout and prepared the final manuscript for typesetting. Proofreading of galleys and pages was handled by Office staff who also handled printing negotiations and managed the distribution of over 3,600 sets of the printed three-volume, 939-page report to the vision research community and the public. About 1,900 of these sets were distributed at the annual meeting of the Association for Research in Vision and Ophthalmology (ARVO), held in Sarasota, Florida. Subsequent to the meeting, return postcards were mailed to all members of ARVO who attended asking if in the event they had not picked up a set in Sarasota they would like copies of the report, or if they had if they would like extra copies. Of 500 postcards sent out, more than 200 were returned requesting one or more sets. Copies of the three volumes were also mailed to ARVO members who did not attend the meeting and to those members of the Association of University Professors of Ophthalmology who are not ARVO members.

Volume One of the report consists of a summary of the Council's research priorities and proposed five-year budget for the NEI, summaries of six panel reports which make up Volume Two, as well as chapters on the background for vision research planning, implementation or program plans, and program and management issues related to vision research planning. Also presented are a data summary and a general discussion of the place of vision research in biomedical science.

Volume Two presents the complete reports of six panels of vision scientists which assayed the state of the art in the five NEI programs and in vision research training. Each program report includes a statement of the problem, review of accomplishments, list of program goals and objectives, discussion of research needs and opportunities in each subprogram, and designation from

these of program priorities. Each report is followed by a set of resource tables which list by subprogram the Council's recommended number of projects for each priority and the financial and manpower resources that will be required to implement them.

Volume Three presents the background information used by the panels concerning project data on ascertainable support of vision research by the NEI, other Government organizations, and national private and philanthropic organizations.

Also in 1978, the Office coordinated preparation of this Annual Report and had primary responsibility for developing and writing NEI's contribution to the NIH Forward Plan for Health and the NIH Evaluation Plan. The Office answered Congressional and Departmental requests relating to NEI planning activities as well as similar inquiries from other Institutes of NIH and organizations and members of the general public. In addition, the Office assisted the Program Information Section, Extramural and Collaborative Programs, in responding to specialized requests for program information including the determination of NEI support of research related to prevention, marine science, Indian health, diabetes, and nutrition.

Dr. Stephen Gordon, an NIH Grants Associate, spent a one-month assignment with the Office developing a prototype system for tracking the implementation of program priorities established in the new five-year vision research plan. After consultation with staff of the Extramural and Collaborative Programs, Dr. Gordon developed a proposal for classifying incoming grant applications according to their relevance to the priorities identified in the planning document. Efforts are now being made to develop this concept further and establish a workable means of monitoring the response of the vision research community to the National Advisory Eye Council's research priorities. Dr. Gordon also investigated the question of research workshops, how they are best planned and organized, and wrote a report of his findings.

Mr. Morris was designated as a member of a small workgroup of B/I/D planning officers to assist the Office of Program Planning and Evaluation, NIH, in its extensive preparations for a National Conference on Health Research Principles, to be held October 3-4 at NIH. This conference is in response to a directive from Secretary Califano for the development of guidelines which could form the basis of a five-year HEW plan for health research. Following the Conference, the working group will assist in preparing the final report which incorporates public testimony and commentary on a draft set of principles with that of the five panels of scientific experts around whose deliberations the Conference will be centered: Fundamental Research, Clinical Applications and Health Services Research, Health Regulation and Promotion, Research Capability, and Unifying Concepts.

## Scientific Reporting

The year was highlighted by a number of special activities, including the production of an exhibit for HEW's 25th Anniversary, projects related to the First General Assembly of the International Agency for the Prevention of Blindness, and significantly increased communications with the press and other news media concerning the NEI's programs and related subjects and issues in the field of eye care.

### Scientific Communications

The Office assisted in activities associated with two major scientific meetings during the year. For the Second Congress of the International Society of Neuro-Ophthalmology, which met at NIH in May, OPPSR staff helped prepare an exhibit at the National Library of Medicine on milestones in the history of neuro-ophthalmology. This exhibit, which was videotaped by the NLM, was well-received by the delegates to the Congress.

Mr. Morris assisted the Institute Director, Dr. Kupfer, in the latter's capacity as Chairman of the Projects and Priorities Committee of the International Agency for the Prevention of Blindness at the IAPB's First General Assembly held in Oxford, England, July 5-7. Mr. Morris helped plan the strategy for the meeting, the purpose of which was to encourage the world's governments to undertake specific programs of action against preventable blindness. He also provided a detailed report of the final plenary session for use in preparing the Assembly's official report.

The Office assisted the Director in the preparation of speeches, including presentations before the IAPB's First General Assembly, the annual meeting of the National Society for the Prevention of Blindness, the Society for Neuroscience, and the dedication of the Cullen Eye Institute at Baylor College of Medicine in Houston. Speech materials were also assembled for the Director, NIH, for a planned address to be given to the American Academy of Ophthalmology.

For a symposium entitled "Focus on Progress" held in conjunction with the dedication of the Cullen Eye Institute, Mr. Morris, substituting for Dr. Kupfer, made a presentation on the role of government in eye research. The symposium was attended by over 200 allied health workers in the field of eye care.

The Office coordinated and edited the submission of material to be included in three NIH Special Reports to Congress: Allergy and Organ Transplantation, Diabetes, and Genetic Diseases. These stressed the contribution of vision research to each of these fields during the past year.

The Office assisted the Office of Biometry and Epidemiology in planning for the publication of a monograph on the Framingham Eye Study by taking part in negotiations with the editorial staff of the American Medical Association, which will publish the monograph as a supplement to the journal, Archives of Ophthalmology.

As in previous years, the Office drafted Dr. Kupfer's Opening Statement before the House and Senate Appropriations Committees and helped review and edit the transcripts of these hearings.

The Office assisted the Diabetic Retinopathy Study Group and the NEI Office of Biometry and Epidemiology in making extensive revisions in two widely-distributed and utilized physician-oriented booklets on the Diabetic Retinopathy Study (DRS) and Diabetic Retinopathy Vitrectomy Study (DRVS). These booklets are intended to foster the application of the DRS findings to clinical practice and to communicate the goals and objectives of the DRVS to ophthalmologists and other interested health professionals for the purpose of aiding patient recruitment.

The Office also assisted the Scientific Programs Branch, Extramural and Collaborative Programs, in the preparation of two booklets: Guidelines for National Eye Institute Core Grants for Vision Research Centers and Directory of National Eye Institute Institutional Fellowships for Vision Research Training.

#### Consumer Education

For HEW's 25th Anniversary celebration held May 23-24 at the Hubert H. Humphrey Building in Washington, D.C., the Office prepared an NEI exhibit which stressed the accomplishments of vision research over the past 25 years, provided an opportunity for visitors to the HEW celebration to have their vision tested, and illustrated how various eye conditions can affect vision. Updated NEI fact sheets on various eye disorders were prepared for distribution at the exhibit site, which attracted an estimated 3,500 to 5,000 visitors for each of the two days. The exhibit was staffed by nine NEI staff members--four ophthalmologists and three technicians from the Clinical Branch and two members of the OPPSR staff--on a rotating basis. Those staffing the exhibit thought that the display had a most favorable impact on the public and in return provided a valuable opportunity for the staff to learn first-hand about the kinds of eye problems that concern the public. Both Secretary Califano and Assistant Secretary for Public Affairs, Eileen Shanahan, visited the exhibit.

At the request of NIH's Division of Public Information, Audiovisual Branch, the Office prepared background information to be used in soliciting a contract proposal to produce NIH radio and television public service announcements on cataract. The intended message would be that cataract patients can have visual function restored in the great majority of cases and that anyone who has been advised by an ophthalmologist to have cataract surgery should seriously consider doing so. A contractor for this project has been selected by NIH, and the Office will help supervise the production of these spot announcements during the coming year. In the meantime, previously used NIH public service announcements cataract, glaucoma, and the importance of regular eye examinations were revised and distributed by NIH to 1,000 radio stations.

The Office initiated contact with producers of the new public television series, "Over Easy," to determine their interest in presenting information on vision research and eye care for the elderly. In the course of discussions with program officials, we suggested a number of individuals who might be suitable for interviewing on the show. These included Dr. Bruce Spivey, Executive Vice President of the American Academy of Ophthalmology, who subsequently was contacted by "Over Easy" and agreed to appear on the show. Dr. Spivey discussed eye disorders associated with aging on two programs that were broadcast in January and February to 261 public television stations across the country. In addition, NEI provided fact sheets to the program for use in responding to viewers who asked for more information.

### Press Relations

Contacts between the Office and the news media increased significantly during the year. Publications, electronic media, and news services which contacted us include U.S. Medicine, the Associated Press, Medical World News, Science News, National Enquirer, Scripps-Howard News Service, New York Magazine, NBC Television, CBS Television, Modern Medicine, Better Homes and Gardens, The Washingtonian, Reader's Digest, Diabetes Outlook, Houston Magazine, WRC Radio, New Republic, Smithsonian Magazine, U.S. News and World Report, Peter Weaver (syndicated consumer columnist) and a number of local newspapers. Several inquiries were received from freelancers preparing books, articles, and scripts. Manuscripts were submitted to the Office for review in advance of publication by Vogue, Good Housekeeping, Better Homes and Gardens, and Patient Care. Better Homes and Gardens also asked the Office to review the eye section of their Family Guide to Health to indicate topics that needed updating in a planned revision of this book. Photographs were provided to Pharmacy Times, Surgical Rounds, Aging Magazine (published by HEW's Administration on Aging), Encyclopedia Britannica Medical Annual, a textbook Bioscope, and Omni, a new science fact-fiction magazine. Major articles mentioning the National Eye Institute appeared during the year in McCall's, The Blue Sheet, New Republic, Science Digest, Harper's Bazaar, American Optometric Association News, Boston Globe, Miami Herald, Good Housekeeping, American Medical News, Journal of the American Medical Association, New York Daily News, Medical World News, New York Times, Medical Tribune, Minneapolis Tribune, Family Circle, Hospital Tribune, and Newsday. As a result of one article which appeared in the New York Times on cataract, the NEI received more than 4,000 individual requests for its booklet, Cataract--Focus on Research. We were able to respond to this unexpected deluge in a reasonable period of time only because of the fine help and support of the Administrative Officer (OD) and staff of the Program Information Section, Extramural and Collaborative Programs.

Search for Health columns, a monthly NIH service for weekly newspapers, were prepared on amblyopia, diabetic retinopathy, cataract, macular degeneration, and corneal disorders. News releases or announcements were issued on the appointment of new National Advisory Eye Council members, the awarding of contracts for the Early Treatment of Diabetic Retinopathy Study, the collaboration between NEI and NASA on the development and testing of a new cataract extraction device, the awarding of a contract to evaluate the feasibility of

analyzing ophthalmologists' records of intraocular lens implantation, and the release of the report, Vision Research--A National Plan: 1978-1982.

### Controversy: Fact or Fiction

One indication of the public's great interest in eye disorders and blindness is the many letters we receive in response to incomplete or possibly misleading information about eye research and new forms of treatment that appear from time to time in the news media.

Early in FY 1978, in response to an advertisement in a widely circulated health magazine, the National Eye Institute received over 70 letters from people across the country asking where they could obtain flavonoid compounds for treating cataract. One small section of the advertisement had stated that NEI research showed that certain of these compounds could retard formation of cataract. Unfortunately, the advertisement neglected to explain that these findings were applicable to only experimental sugar cataracts in rodents and had no clinical application at the present time. With the assistance of Dr. Jin Kinoshita and Dr. Shambu Varma, the Office prepared a fact sheet on NEI cataract research with bioflavonoids which explained the purpose and status of this research. This was sent to those who asked for information on this subject.

In the spring and summer of 1978, news reports of a Soviet therapy for retinitis pigmentosa, a disease for which there is currently no accepted treatment in the United States and most western nations, resulted in many letters from retinitis pigmentosa patients asking how they could obtain this treatment. NEI's Clinical Director, Dr. Elmer Ballintine, who had attended an international scientific meeting on this treatment in Moscow in December 1977, reported that the results of the Soviet treatment as presented at the meeting were inconclusive and that further research was required. Dr. Ballintine's scientific report was edited by Office staff for use in responding to a steady number of inquiries on this subject.

In June 1978, a wire service story reported that the Soviets were using the Q-switched laser for treating cataract and glaucoma. This story resulted in many public inquiries from people who wanted to come to the NEI for laser surgery because the article mentioned that NEI was conducting research with the Q-switched laser. Fact sheets on glaucoma and cataract and an explanation of the current status of NEI research with the Q-switched laser were sent in response to these inquiries.

In May, the Associated Press reported that animal experiments at the NIH suggested that prolonged use of acetaminophen, an aspirin substitute, may trigger formation of cataract in some humans. The story, based on a report in the journal Science, was carried by 451 newspapers and was discussed on nationwide radio and television. Medical World News and Medical Tribune carried follow-up stories. Many of the press accounts included a statement by Dr. Kinoshita disclaiming the relevance of this research to human cataracts. The OPPSR worked with Dr. Kinoshita to prepare a fact sheet to respond to further inquiries. The experience underlined the importance of trying to

anticipate press interest in NEI scientific papers, particularly those whose findings may be controversial or subject to misinterpretation.

A subject of increasing interest to the press and the public is the potential use of marihuana and its derivatives in the treatment of glaucoma. This subject encompasses an amalgam of social, legal, and scientific issues and questions, and to help clarify the latter aspects, the Office also prepared a fact sheet on this subject for use in responding to public, press, and Congressional inquiries. The fact sheet has also been adopted by the HEW Interagency Committee on New Therapies for Pain and Discomfort.

### Public Inquiries

Through an intense effort on the part of the entire information staff of the Office, a large backlog of public inquiries was eliminated toward the end of the year, and a new Office correspondence control system was instituted to insure a maximum two-week turnaround for most public inquiries. It is expected that this system, by helping keep correspondence at a manageable level, will free staff for other projects, including the preparation of more extensive printed public information materials for use in answering public inquiries.

During the past year, over 900 letters from the public were received which required individual attention and response. In addition, 23 written Congressional inquiries and 18 additional letters controlled through the NIH Executive Secretariat were answered. Much of the latter was correspondence addressed to the Secretary of Health, Education, and Welfare. At least 1,200 telephone calls were handled by our information specialists during the year. Of these calls, approximately 50 were from Congressional offices and 75 were from the news media or freelance writers. Another 100 or more asked for statistical information on the incidence, prevalence, and costs of eye disorders or on NEI's expenditures for vision research. An additional 1,200 calls were screened by secretarial and clerical staff and either handled routinely by them or referred elsewhere.

Some of the most commonly asked questions were the following:

1. What are the new techniques for cataract removal, and where are they available?
2. What are the differences between soft and hard contact lenses, and what are some of the new types of contact lenses being developed, especially those for long-term wear?
3. What does the National Eye Institute think of intraocular lens implantation? Can a person who has already had cataract surgery have a lens implant? If I have glaucoma (or diabetes), can I have a lens implant when I have cataract surgery?
4. Is there any treatment for retinitis pigmentosa? Is there likely to be one in the near future? What about the Soviet treatment that has been widely publicized?

5. What treatment is available for macular degeneration? Is NEI conducting research on this? Who else is doing so?
6. What does NEI think about the "X-Chrom" lens? Does it really help people who are color deficient? Where is the man located who developed this lens?
7. What is presumed ocular histoplasmosis? How is it treated, and who is conducting research on it?
8. How does a person apply for treatment at the NEI? What diseases are the doctors investigating at NEI? Can I have a cataract operation at NIH?
9. Can radar or visual display computer terminals cause cataract?
10. Are lasers used to remove cataracts?
11. How is glaucoma surgery performed?
12. Is glaucoma hereditary?
13. Where are the best centers for treating glaucoma?
14. Can a person with glaucoma have cataract surgery or lens implants?
15. What are the side effects of glaucoma drugs?
16. Where can I get marihuana to treat glaucoma?
17. Can Q-switched lasers be used to treat glaucoma in this country like they are being used in the Soviet Union?
18. Do you have any statistics on the incidence and prevalence of glaucoma?
19. Where can I get photocoagulation treatment for diabetic retinopathy?
20. How many Americans wear eyeglasses or contact lenses?

## Publications

The Office distributed the following number of publications during the year:

### Consumer Information

Cataract -----	6,532
Corneal Diseases -----	557
Diabetic Retinopathy -----	963
Glaucoma -----	1,546
Macular Degeneration -----	685
Refractive Errors -----	573
Retinal Detachment -----	298
Retinitis Pigmentosa -----	667
Know Your Eyes -----	510

### Professional and Scientific

Statistics on Blindness in the Model Reporting Area, 1969-1970 -----	27
Evaluation of the Treatment of Diabetic Retinopathy, A Research Project, Reprint from the Sight- Saving Review -----	62
Vision Research Program Planning -----	125
Support for Vision Research -----	128
Summary and Critique of Available Data on the Prevalence and Economic and Social Costs of Visual Disorders and Disabilities (Westat Report) -----	27
Vision Research--A National Plan: 1978-1982 (total sets of Volumes One, Two, and Three) -----	3,600
TOTAL	16,304

## Miscellaneous

The Office, as in past years, drafted the annual Presidential proclamation for Save Your Vision Week (March 5-11, 1978) and White Cane Safety Day (October 15, 1978). Messages of greetings were also prepared at the request of the White House for the First General Assembly of the IAPB and the meeting of the International Optometric and Optical League. Information was supplied to the Director, NEI, for his contribution to Documenta Ophthalmologica's special issue in honor of Dr. John Harris. Office staff had a plaque prepared in honor of Dr. Sidney Futterman's contributions to NIH and vision research. An NIH Record obituary on Dr. V. Everett Kinsey was prepared. An Office staff member is representing the NEI on a committee planning an NIH exhibit marking the International Year of the Child.

Mr. Morris was designated Chairman of an NIH Publications Study Group. The Group, consisting of five other NIH Information Officers, was formed in response to Congressional criticisms of NIH nutrition publications but has decided to look broadly into the entire publications process at NIH. As its first activity, the Group prepared and sent a detailed questionnaire to all B/I/D Information Offices designed to gather information and expert opinions on NIH publications: how publications originate and are conceived, prepared, reviewed, designed, cleared, disseminated, and kept up to date. The Group's activities will continue into FY 1979.

Mr. Morris was also invited to serve as a member of the Public Relations Task Force of the National Society for the Prevention of Blindness which met in New York in November 1977. The Task Force made recommendations to the NSPB on improving its public image and identification.

INTRAMURAL RESEARCH



ANNUAL REPORT  
NATIONAL EYE INSTITUTE  
October 1, 1977 - September 30, 1978

REPORT OF THE DIRECTOR OF INTRAMURAL RESEARCH  
Carl Kupfer, M.D.

The intramural research program at the National Eye Institute serves many purposes. Although its primary function is to contribute to the national vision research effort, the intramural program also helps insure that the concerns, viewpoints, and technical expertise of vision research scientists are taken into account in policy decisions at the NEI. In addition to the accomplishments reported in the following pages, the contributions of NEI intramural scientists to the management of the Institute during the past year include participating in the planning and running of research workshops, serving as consultants to the National Advisory Eye Council during the preparation of Vision Research--A National Plan: 1978-1982, assisting in the response to inquiries from the public concerning NEI programs, and serving as representatives of the NEI on special commissions and committees.

A significant accomplishment in the NEI's Clinical Branch during the past year was the consistent growth in tissue culture of trabecular meshwork from monkey and human eyes. This new technique is proving useful in biochemical and physiological studies of glaucoma. Tissue culture of corneal cells has permitted the study of the effects of various growth factors on corneal metabolism, work which is relevant to the understanding of corneal wound healing. The toxicity of antiglaucoma drugs on corneal tissue is also being studied by means of this culture.

Other toxicity studies have demonstrated the ocular effects of therapy for hyperkeratinizing dermatologic disorders as well as the retinal toxicity of tomoxifen, a new anticancer drug used in the therapy of metastatic breast carcinoma.

Evidence has been obtained of the possible role of prostaglandins in ocular neovascularization. Plans are now in progress to test the effects of various prostaglandin inhibitors to determine if they are capable of blocking new blood vessel formation.

Studies to improve the diagnosis of intracranial disease by characterizing ocular motor disturbances and to learn more about the role of various portions of the brain in the control of eye movements have shown the surprising finding that the cerebellum processes visual information rather than simply modulating the motor control of eyes. This is especially significant because the cerebellum has been generally regarded solely as a motor organ.

Investigations of laser-induced chronic glaucoma in the rhesus monkey have included morphologic studies of cross-sections of the optic nerve and retina which has led to the identification of the scleral lamina cribrosa as

a site of blockage of axoplasmic transport in this animal model. A model for ocular hypotony has also been developed in rhesus monkeys, and studies are in progress to demonstrate the effect of medications. An artificial aqueous humor has been prepared and tested as part of a search for less destructive perfusates for use in ocular surgery such as vitrectomy.

In ongoing studies of the pharmacodynamics of various agents affecting the intraocular pressure, prostaglandins have been shown to decrease aqueous humor production, and a rational mechanism has been proposed to describe various intraocular pressure effects of prostaglandins.

A new instrument, the chromagraph, has been developed which is capable of measuring any type of color vision defect, many of which are too subtle or bizarre to be shown with conventional tests. The biochemical basis for corneal transparency and opacification has been further elucidated, and clinicopathologic correlative studies of human corneal dystrophies have provided new information which promises to improve the diagnosis and classification of such disorders and lead to more rational therapy.

The first evidence of the presence of a specific enzyme effect in gyrate atrophy of the retina was reported last year. This is the first confirmation of an enzyme defect in an inherited retinal degenerative disease, and based on this finding, a study of specific therapy for this disorder will proceed. This discovery raises hope that similar defects may be found in other retinal degenerative disorders, particularly those which cause progressive visual loss from early childhood.

Perhaps some of the most interesting research performed in NEI's Laboratory of Vision Research during the past year concerned vitamin A, whose derivative plays a key role in vision. Scientists working at the LVR are examining various aspects of vitamin A metabolism in the retina and cornea in an effort to gain insight into clinical problems associated with vitamin A abnormalities. New findings suggest that vitamin E also plays a vital role in maintaining the normal state of ocular tissues.

As an outgrowth of studies of the role of aldose reductase in the development of metabolic cataracts, it has been found that this enzyme is also present in the corneal epithelium of the bovine eye. This finding may have relevance to the pathogenesis of corneal changes that occur in diabetics. Research on cataract was furthered by the development of successful tissue culture techniques which can be used to study congenital and hereditary lens opacities. The cultured cells apparently retain the traits of cells in the original tissue and therefore may help in elucidating the mechanisms that initiate the cataractous process. Clinicopathologic correlative studies of human senile cataracts have shown that cytologic changes in the epithelium preceded the occurrence of senile changes in lens fibers.

Continuing studies of visual pigments demonstrated that rhodopsin phosphorylation takes place primarily in the newly formed discs and plasma membrane of the rod. This suggests the possible role of this reaction in disc formation and/or function. A method of introducing dyes into cells which

makes it possible to measure intracellular ionic activity has been developed. This promises to make possible the study of the visual process, including the role of calcium in photoexcitation and the regeneration of visual pigment, in living retinal cells.

Further details of these and other studies in the NEI's intramural program may be found in the following reports of the Clinical Branch and Laboratory of Vision Research.



Clinical Branch





ANNUAL REPORT  
NATIONAL EYE INSTITUTE  
October 1, 1977 - September 30, 1978

REPORT OF THE CLINICAL DIRECTOR  
Elmer J. Ballintine, M.D.

The primary mission of the NEI Clinical Branch is to conduct research related to those aspects of ocular disease which can be studied best in man. Such investigations must meet the same standards of scientific rigor and validity that apply to other biologic experiments and do so within the ethical and humanitarian constraints imposed by the fact that the subjects are people.

Each research plan is reviewed by a protocol review committee composed of representatives from the Clinical Branch, other parts of the NEI and NIH, and others who are not employees of NIH. The protocol may also be reviewed by the Clinical Review Committee of the Clinical Center's Medical Board. These reviews insure that adequate safeguards for rights and welfare of patients are maintained and that patients are fully informed about both risks and potential benefits of their participation. Patients are accepted only if referred by an ophthalmologist outside the Institute and only if the patient's condition is appropriate for study in an approved protocol.

These protocols define the characteristics of patients to be admitted for study, define those features which exclude patients because they are unsuitable for the study, and describe in detail what will be done and, insofar as possible, the methods to be used to interpret the data. When possible, an estimate of the number of patients needed for each study is made, and appropriate methods are used for masking of the data to avoid bias in interpretation. At present, 17 protocols are in operation, and three are awaiting approval.

Study of human eye disease often requires extensive development of laboratory methods and demonstration of biochemical or physiologic mechanisms preliminary to work with patients. Eleven such projects are underway. Other laboratory investigations use material obtained from surgical, autopsy, or blood specimens. Three studies using human tissues are underway.

The Pathology Laboratory processed and examined histopathologically 75 eyes from the autopsy service of the Clinical Center. 1,459 inpatient and 2,567 outpatient consultations were furnished for other NIH Institutes at the Clinical Center. There were 4,026 Clinical Branch outpatient visits, 69 inpatient admissions, and 27 surgical operations were performed.

The Clinical Branch continued to cooperate with other NIH Institutes in the pursuit of unique research opportunities. The study of diabetic retinopathy among the Pima Indians in a project administered by the Epidemiology and Field Studies Branch of the National Institute of Arthritis, Metabolism and Digestive Diseases was continued as was the study of microangiopathy among patients with acromegaly. The study of ocular metastasis in National Cancer Institute patients undergoing treatment of breast carcinomas continued.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00150-05 CB
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less)  Ocular Hypertension Study		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  PI: Elmer J. Ballintine M.D. Clinical Director CB NEI Other: Douglas E. Gaasterland M.D. Senior Staff Ophthalmologist CB NEI Richard Weiblinger B.S. Biologist CB NEI		
COOPERATING UNITS (if any)  Office of Biometry and Epidemiology, NEI		
LAB/BRANCH Clinical Branch		
SECTION		
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20014		
TOTAL MANYEARS: 1.0	PROFESSIONAL: 0.6	OTHER: 0.4
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)  Patients with <u>ocular hypertension</u> are randomly assigned to treatment with topical <u>pilocarpine</u> in one or both eyes or to no treatment. The objectives of the study are: 1) to determine if treatment with pilocarpine to reduce intraocular pressure before visual field changes occur will reduce the number of ocular hypertensive subjects who eventually become glaucomatous, and 2) to determine if measurements of aqueous humor dynamics, the response to water loading of diurnal variation in intraocular pressure, serial stereophotographs of the optic disc, and measurements of visual fields help to predict which patients will eventually become glaucomatous.		

Project Description:

Objectives: Prolonged observation of a series of patients with ocular hypertension, some of whom are treated with miotics, will help to determine which signs have value in predicting those who will eventually require treatment and to determine if early treatment of ocular hypertension has any value in preventing visual field loss or in slowing the rate of development of abnormalities of aqueous humor dynamics.

Methods Employed: A detailed plan for classifying patients with ocular hypertension; observing them by repeated examinations including measurement of visual fields, aqueous humor dynamics, and photogrammetry of the optic disc over a period of five or more years; and randomly assigning patients to treatment with pilocarpine collyria in one or both eyes, or to no treatment, has been standardized.

Major Findings: There has been no indication that the course of ocular hypertension has been affected by treatment. Fifty-two patients have been enrolled to date.

Significance to Biomedical Research and the Program of the Institute: Early, precise identification of patients who require treatment because they are in the early stages of the simple glaucoma remains an unsolved problem. The data being collected in this study will furnish a basis for establishing criteria for treatment more precisely than is now possible. There is at present no detailed knowledge of the progression of optic disc changes in ocular hypertension. The data being collected in this study, as well as the development of better instruments for the measurements in this study, will supply needed information in this field.

Proposed Course: It is expected that the project will continue for at least five years, and we expect to enroll 100 subjects.

NEI Research Program: Glaucoma

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00017-04 CB
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PERIOD COVERED.  
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)  
  
Tissue Culture of Trabecular Meshwork

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Elmer J. Ballintine	M.D.	Clinical Director	CB	NEI
Other:	Richard A. Stone	M.D.	Clinical Associate	CB	NEI
	Richard Weiblinger	B.S.	Biologist	CB	NEI

COOPERATING UNITS (if any)  
  
None

LAB/BRANCH  
Clinical Branch

SECTION

INSTITUTE AND LOCATION  
National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 1.0	PROFESSIONAL: 0.5	OTHER: 0.5
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CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS      ☒ (b) HUMAN TISSUES      ☐ (c) NEITHER

☐ (a1) MINORS    ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Slices of trabecular meshwork from normal monkey eyes and from surgical trabeculectomy specimens from human glaucomatous eyes are being grown in tissue culture. Attempts are being made to identify the tissue of origin of the resulting cellular growth and to grow trabecular endothelial cells selectively.

Differences between trabecular tissues from glaucomatous and normal human eyes with respect to details of growth and metabolic activity are being sought.

Project Description:

Objectives: Much evidence indicates that in simple open-angle glaucoma, the obstruction to aqueous humor outflow lies within the trabecular meshwork and the inner wall of Schlemm's canal. The amounts of human trabecular tissue available for biochemical and physiologic study are insufficient for most in vitro research methods. Therefore, tissue culture techniques are being employed in the hope of developing a system in which the basic physiologic and biochemical abnormality present in open-angle glaucoma can be explored. After a satisfactory culture system is developed, various metabolic and physiologic activities of the cultured cells will be explored.

Methods Employed: Specimens of trabecular tissue are obtained from monkey eyes for preliminary studies. Some surgical specimens from patients undergoing trabeculectomy have been studied. More of these surgical specimens as well as controls from human autopsy eyes are being sought.

Specimens of trabecular meshwork are sectioned into small fragments under the dissecting microscope and placed in tissue culture medium. Phase contrast microscopy is used to observe growth and form of these cells. They are being further characterized by their histologic and histochemical properties. Methods and criteria for growing trabecular epithelial cells free of fibroblasts are being developed.

Major Findings: Trabecular meshwork from monkey and human eyes has been grown consistently in tissue culture, and the conditions for this growth have been determined. It has been possible to obtain some cultures without a significant fibroblastic contamination.

Significance to Biomedical Research and the Program of the Institute: The mechanism by which the resistance to aqueous humor outflow increases in open-angle glaucoma is at present unknown. This project may be able to define the physiologic and biochemical abnormalities of trabecular epithelium that are the fundamental causes of open-angle glaucoma.

Proposed Course: Metabolic studies of the cultured cells will attempt to demonstrate differences in how they synthesize collagen and mucopolysaccharide between normal cultures and those from human eyes that have a pressure elevation following administration of topical corticosteroids.

NEI Research Program: Glaucoma--Etiology of Glaucoma (Primary Open-Angle Glaucoma)

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00022-04 CB																								
PERIOD COVERED October 1, 1977 to September 30, 1978																										
TITLE OF PROJECT (80 characters or less)  Urokinase Central Retinal Vein Occlusion Trial																										
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																										
<table style="width: 100%; border: none;"> <tr> <td style="width: 30%;">PI:</td> <td style="width: 30%;">Elmer J. Ballintine</td> <td style="width: 10%;">M.D.</td> <td style="width: 20%;">Clinical Director</td> <td style="width: 10%;">CB</td> <td style="width: 10%;">NEI</td> </tr> <tr> <td>Other:</td> <td>David C. Allen</td> <td>O.D. M.A.</td> <td>Statistician</td> <td>OBE</td> <td>NEI</td> </tr> <tr> <td></td> <td>Harvey R. Gralnick</td> <td>M.D.</td> <td>Chief, Hematology Service</td> <td>CC</td> <td>NIH</td> </tr> <tr> <td></td> <td>Richard Weiblinger</td> <td>B.A.</td> <td>Biologist</td> <td>CB</td> <td>NEI</td> </tr> </table>			PI:	Elmer J. Ballintine	M.D.	Clinical Director	CB	NEI	Other:	David C. Allen	O.D. M.A.	Statistician	OBE	NEI		Harvey R. Gralnick	M.D.	Chief, Hematology Service	CC	NIH		Richard Weiblinger	B.A.	Biologist	CB	NEI
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Other:	David C. Allen	O.D. M.A.	Statistician	OBE	NEI																					
	Harvey R. Gralnick	M.D.	Chief, Hematology Service	CC	NIH																					
	Richard Weiblinger	B.A.	Biologist	CB	NEI																					
COOPERATING UNITS (if any)  Office of Biometry and Epidemiology, NEI																										
LAB/BRANCH Clinical Branch																										
SECTION																										
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20014																										
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:																								
0.3	0.2	0.1																								
CHECK APPROPRIATE BOX(ES)																										
<input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER																										
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																										
SUMMARY OF WORK (200 words or less - underline keywords)																										
<p>Patients with recent complete <u>occlusion</u> of the <u>central retinal vein</u> are randomly assigned to treatment either with intravenous urokinase followed by heparinization alone, or treatment with intravenous fluids alone. The patients are then examined periodically for one year, and the effectiveness of treatment is judged by restoration of vision and the degree of protection achieved against the development of <u>hemorrhagic glaucoma</u>.</p>																										

Project Description:

Objectives: To determine if treatment with a thrombolytic agent (Urokinase) plus anticoagulation with heparin, or treatment by anticoagulation with heparin alone is effective in reducing the loss of visual acuity and the progression to hemorrhagic glaucoma that is a consequence of occlusion of the central retinal vein.

Methods Employed: Patients are examined according to a detailed plan for eligibility in the study. Eligible patients, if they agree to participate, are assigned by randomization to one of three treatment plans:

1) Twenty-four hours of continuous intravenous treatment with urokinase in an effort to resolve the occlusion of the central retinal vein. This is followed by two weeks of anticoagulation treatment with heparin to prevent reformation of venous obstruction.

2) Heparin anticoagulation alone.

3) Hospitalization and administration of intravenous fluids similar in volume to those used in the other treatment groups.

After the treatment period, the patients are examined periodically for one year to determine the rate at which hemorrhagic glaucoma occurs and the degree of restoration of vision to the eye.

Major Findings: The protocol has been perfected, and six patients have been randomized to treatment. No trends have been observed.

Significance to Biomedical Research and the Program of the Institute: Occlusion of the central vein is a serious cause of visual disability, and one of its major consequences is hemorrhagic glaucoma, which almost invariably results in a blind, painful eye. In the past, treatment with anticoagulation has been advocated, but no convincing evidence of effectiveness has been published. With the development of an effective thrombolytic agent (Urokinase), the possibility of dissolving the presumed cause of the obstruction, a thrombus in the central retinal vein, and the demonstration that urokinase is effective in thrombolytic disease in other sites support the decision to undertake this trial.

Proposed Course: Examination of published data on the course of occlusion of central retinal vein indicates that it will require recruitment of 75 patients to demonstrate that a 50% improvement in visual results is produced by the treatment. We will continue to recruit until 75 patients have been treated.

NEI Research Program: Retinal and Choroidal Diseases--Diabetic Retinopathy and Other Vascular and Circulatory Abnormalities

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00080-01 CB										
PERIOD COVERED October 1, 1977 to September 30, 1978												
TITLE OF PROJECT (80 characters or less)  Cornea: Growth Factors and Corneal Cell Metabolism												
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: David BenEzra</td> <td style="width: 33%;">M.D., Ph.D.</td> <td style="width: 33%;">Visiting Scientist</td> <td style="width: 10%;">CB</td> <td style="width: 10%;">NEI</td> </tr> <tr> <td>Other: Sandra Bornstein</td> <td></td> <td>Technician</td> <td>CB</td> <td>NEI</td> </tr> </table>			PI: David BenEzra	M.D., Ph.D.	Visiting Scientist	CB	NEI	Other: Sandra Bornstein		Technician	CB	NEI
PI: David BenEzra	M.D., Ph.D.	Visiting Scientist	CB	NEI								
Other: Sandra Bornstein		Technician	CB	NEI								
COOPERATING UNITS (if any)  None												
LAB/BRANCH Clinical Branch												
SECTION												
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20014												
TOTAL MANYEARS: 0.3	PROFESSIONAL: 0.1	OTHER: 0.2										
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS												
SUMMARY OF WORK (200 words or less - underline keywords)  The influence of growth factors on the corneal cell metabolism was studied using the microculture technique. Both the <u>fibroblast growth factor (FGF)</u> and the <u>epidermal growth factor (EGF)</u> boosted the <u>DNA synthesis in epithelial, stromal, and endothelial cultures</u> in vitro. The <u>nerve growth factor (NGF)</u> , on the other hand, had a very slight influence on stromal cell cultures only.												

Project Description:

Objectives: To investigate the influence of growth factors on the various corneal cell layers and to study the possibility of differentially influencing the proliferation of corneal cells by the various growth factors.

Methods Employed: The microculture method for the assessment of corneal cell metabolism in vitro as developed in our laboratory was used. The growth factors in various dilutions were added to primary and confluent cultures for various lengths of time. The DNA and protein synthesis in the various cultures was assessed at various intervals by the extent of H<sup>3</sup> thymidine or H<sup>3</sup> leucine incorporation.

Major Findings: Both EGF and FGF stimulated markedly the DNA synthesis in initial cultures with low cell density (50 to 100 cells/culture) and in established, nearly confluent cultures. Protein synthesis, on the other hand, was only slightly boosted at the peak of the DNA synthesis phase. Stromal cells were the most sensitive and demonstrated the highest index of stimulation (synthesis with GF/synthesis without) to both EGF and FGF. Human endothelial cultures were less sensitive to the growth factors than the stromal cultures. Established epithelial cultures responded the least and showed a higher index of stimulation with FGF than with EGF. Initial-primary epithelial cultures, on the other hand, responded markedly to both EGF and FGF. The highest index of response was observed to EGF when human corneal epithelial cultures were stimulated by 100 ng/ml. The effect of the growth factors depended on the serum concentration. The highest index of stimulation was obtained in serum concentrations of 1 to 5% or in its absence.

Significance to Biomedical Research and the Program of the Institute: A better understanding of the growth factors' effect on corneal cell proliferation could be beneficial in the management of corneal wound healing.

Proposed Course: To study the effect of EGF, FGF, and NGF on corneal wounds in vivo.

NEI Research Program: Corneal Diseases--Corneal Transplantation and Stromal Injury and Repair

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00054-02 CB																		
PERIOD COVERED October 1, 1977 to September 30, 1978																				
TITLE OF PROJECT (80 characters or less)  Cornea: Organ and Cellular Cultures of Epithelium Stroma and Endothelium																				
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 30%;">David BenEzra</td> <td style="width: 15%;">M.D., Ph.D.</td> <td style="width: 30%;">Visiting Scientist</td> <td style="width: 10%;">CB</td> <td style="width: 5%;">NEI</td> </tr> <tr> <td>Other:</td> <td>Teruo Tanishima</td> <td>M.D.</td> <td>Visiting Scientist</td> <td>CB</td> <td>NEI</td> </tr> <tr> <td></td> <td>Sandra Bornstein</td> <td></td> <td>Technician</td> <td>CB</td> <td>NEI</td> </tr> </table>			PI:	David BenEzra	M.D., Ph.D.	Visiting Scientist	CB	NEI	Other:	Teruo Tanishima	M.D.	Visiting Scientist	CB	NEI		Sandra Bornstein		Technician	CB	NEI
PI:	David BenEzra	M.D., Ph.D.	Visiting Scientist	CB	NEI															
Other:	Teruo Tanishima	M.D.	Visiting Scientist	CB	NEI															
	Sandra Bornstein		Technician	CB	NEI															
COOPERATING UNITS (if any)  None																				
LAB/BRANCH Clinical Branch																				
SECTION																				
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20014																				
TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.3	OTHER: 0.2																		
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																				
SUMMARY OF WORK (200 words or less - underline keywords)  <p>The cornea was dissected under the microscope to its three layers. The in vitro behavior of isolated epithelium, stroma or endothelium as well as the reciprocal influence of these layers in combined cultures was studied.</p> <p><u>Electron microscopic</u> and <u>metabolic</u> studies of <u>epithelial</u> or <u>stromal</u> cultures, on the one hand, and of <u>combined cultures</u>, on the other hand, demonstrated that keratocyte activity was inhibited by the presence of epithelial cells in culture.</p> <p><u>Endothelial membranes</u> consisting of Descemet's membrane and cultured endothelial cells were kept in culture for up to eight weeks and <u>transplanted in vitro</u> onto corneas devoid of Descemet's membrane. Most endothelial membranes retained their characteristics after <u>transplantation</u>.</p>																				

Project Description:

Objectives: To study the possible existence of a regulatory influence between the corneal epithelium and stroma and to test the possibility of producing endothelial membranes that can be kept for a very long period of time in vitro and transplanted.

Methods Employed: The epithelial stromal interaction(s) was studied in corneas obtained from inbred guinea pigs. Epithelial or stromal cultures were prepared according to the method routinely used in our laboratory.

The endothelial membranes were prepared by stripping off Descemet's membrane from rabbit, monkey, or human corneas. These were kept in vitro for various lengths of time and examined periodically by inverted light microscopy and scanning electron microscopy.

Major Findings: Transmission electron microscopy of stromal explants revealed the presence of clear zones around the active keratocytes. Such clear spaces were not observed within the stroma of combined cultures. A possible explanation could be the inhibition of a collagenolytic effect exerted by the epithelial cell metabolism. This postulation is reinforced by the finding that supernatants of epithelial cell cultures inhibited the DNA synthesis of stromal cells.

The behavior of endothelial cells was followed by daily examination under the inverted microscope and by electron microscopy after fixation at various intervals. Endothelial cells from all origins demonstrated a rapid repair mechanism. As early as one hour after the initiation of culture, a marked activity of the endothelial cells five to six cell-diameter distance from the cut edge of the wound is observed. Cells at a farther distance remain morphologically inactive. The active cells round-up, elongate, and mimic a fibroblast-like structure moving to fill bare spaces on the Descemet's. In the process of filling the gaps, the active cells settle and reverse to the characteristic morphological appearance of endothelial cells. These initially are larger; however, mitosis stops only when most cells have reverted to original size. Although the activity on the Descemet's was observed in all species, there was a marked difference in the ability of the endothelial cells to outgrow from the Descemet's. The weakest potential of outgrowth was observed when Descemet's and endothelium from older human corneas were used. Most endothelial membranes retained their characteristics when transplanted to corneas devoid of Descemet's and kept further in organ culture.

Significance to Biomedical Research and the Program of the Institute: The various events in corneal wound healing in the presence or absence of epithelium can be rationalized on the basis of an existing interaction between the two layers of cells.

The results obtained with the endothelial membranes suggest the possibility of transplanting in the endothelium diseases of the cornea where this layer is the only one affected.

Proposed Course: To investigate further the interaction between the various layers of the cornea using other than inbred species and to perform in vivo transplantation of endothelial membranes.

NEI Research Program: Corneal Disease--Corneal Transplantation and Stromal Injury and Repair/Corneal Edema, Dystrophies, and Inherited Disorders

Publications:

BenEzra D, Tanishima T: Possible regulatory mechanisms of the cornea.  
I. Epithelial-stromal interaction in vitro. Arch Ophthalmol (in press).



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00076-01 CB
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PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Effect of Antiglaucomatous Drugs on Corneal Cell Cultures

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David BenEzra	M.D., Ph.D.	Visiting Scientist	CB	NEI
Other:	Robert Nussenblatt	M.D.	Clinical Associate	CB	NEI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Clinical Branch

SECTION

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

0.2

PROFESSIONAL:

0.2

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS

☒ (b) HUMAN TISSUES

☐ (c) NEITHER

☐ (a1) MINORS

☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The relative corneal toxicity of antiglaucomatous drugs used routinely in ophthalmology was assessed in a microculture technique. DNA and protein synthesis of monkey corneal endothelial cell cultures were markedly inhibited by the addition of 25 µg/ml epinephrine, 0.5 mg/ml timolol maleate, or 2.5 mg/ml pilocarpine. Stromal and epithelial cultures were not affected by a concentration of 2.5 mg/ml pilocarpine or 50 µg/ml epinephrine. The metabolism of stromal and epithelial cells was totally inhibited by a concentration of 2.5 mg/ml timolol maleate.

Scanning electron microscopic studies of the above cultures revealed a marked distortion of the cellular structures at the inhibiting concentrations.

Project Description:

Objectives: To study the effect of drugs used for glaucoma therapy on the cell metabolism of the various corneal layers.

Methods Employed: Epithelial, stromal, and endothelial cultures were prepared in a microculture system. Various dilutions of the tested compounds (epinephrine, pilocarpine and timolol) were added. The cell metabolism was assessed by the degree of  $^3\text{H}$  leucine or  $^3\text{H}$  thymidine incorporation.

Major Findings: Concentrations of epinephrine, similar to those used for glaucoma therapy, affect markedly the corneal endothelial cell metabolism in vitro.

Timolol maleate, in high concentrations, is toxic to all layers of the cornea. The endothelial cells were more sensitive than the stromal or epithelial cells.

Pilocarpine was found to be relatively nontoxic in this in vitro system.

Significance to Biomedical Research and the Program of the Institute:  
The treatment of glaucoma with epinephrine or timolol maleate should be carefully monitored for early corneal toxic signs.

Proposed Course: A search for less toxic derivatives will be attempted.

NEI Research Program: Glaucoma--Etiology of Glaucoma (Primary Open-Angle Glaucoma)

Publications:

BenEzra D: A micro culture technique for the evaluation of corneal cell metabolism in vitro. Invest Ophthalmol Visual Sci 16:893, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00081-01 CB
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PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (60 characters or less)

Mediators of Immune Reactions and Ocular Neovasculogenesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David BenEzra	M.D., Ph.D.	Visiting Scientist	CB	NEI
Other:	Sandra Bornstein		Technician	CB	NEI
	James Ingram		Biological Laboratory		
			Technician	CB	NEI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Clinical Branch

SECTION

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

1.5

PROFESSIONAL:

0.5

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Rabbit leukocytes activated in vitro by concanavalin A (Con A) or lipo-polysaccharide (LPS) produce and release to the medium a neovascular attracting factor (NAF). In attempts to elucidate the possible role of some mediators of immune reactions, cyclic nucleotides, prostaglandins, synthetic chemoattractants, and growth factors were extensively studied. Prostaglandin E<sub>1</sub> was the most consistent and the strongest stimulator of new blood vessel proliferation. PGE<sub>2</sub> and PGF<sub>2α</sub> demonstrated a less stimulatory effect while PGF<sub>1α</sub>, PGD<sub>2</sub>, and PGA<sub>1</sub> had no stimulatory potential. The cyclic nucleotides and the synthetic chemoattractants had no NAF potentials. Epidermal growth factor (EGF) demonstrated a moderate neovasculogenetic activity, fibroblast growth factor (FGF) was less stimulatory than EGF, while nerve growth factor (NGF) had no stimulatory potential.

Due to the outstanding and consistent neovasculogenetic effect of PGE<sub>1</sub>, a basic mechanism of neovascularization in which the prostaglandins may fulfill the role of regulators of blood vessel proliferation is suggested.

Project Description:

Objectives: This study was undertaken in order to elucidate the role of the immune mechanism in neovasculogenesis.

Methods Employed: Leukocyte Cultures-- $10^5$  mononuclear cells were seeded per culture in micro titer plates and stimulated by Con A or LPS. Twenty-four hours after the stimulation, supernatants or activated cells were injected into rabbit corneas. The growth of blood vessels toward the injection site was observed under the operating microscope during a period of six days. At the end of the observation period, eyes were removed for histology.

Corneal Implants--Midstromal implants consisting of ethylene-vinyl-acetate copolymer (40% vinyl-acetate by weight)--Elvax 40--sequestering various concentrations of dry material to be tested were used. These implants are positioned 2 to 3 mm from the limbus, in the midstroma, under the operating microscope.

Major Findings: Nonactivated leukocytes or their culture supernatants did not demonstrate any neovascular activity. Con A and LPS activated cultures demonstrated the release of NAF as evidenced by the potential of these cultures to induce neovasculogenesis.

Synthetic chemoattractants and cyclic nucleotides had no neovasculogenetic activity. Among the growth factors, epidermal growth factors, in most of the cases, induced a moderate blood vessel proliferation while fibroblast growth factor (FGF) was a milder stimulant. Nerve growth factor (NGF) was nonvasculogenic.

The strongest and most consistent NAF activity was demonstrated by  $PGE_1$ .  $PGE_2$ , and  $PGF_{2\alpha}$  were less vasculogenic while  $PGF_{1\alpha}$ ,  $PGA_1$ , and  $PGD_2$  did not show any significant neovasculogenetic activity.

Significance to Biomedical Research and the Program of the Institute: If ocular neovasculogenesis is mediated and/or regulated by the local production and release of prostaglandins, treatment with inhibitors of prostaglandin synthesis might be beneficial.

Proposed Course: To test the effect of inhibitors of prostaglandin synthesis on ocular neovasculogenesis as produced experimentally in this system and to test the possible regulatory effect of other prostaglandins or derivatives on ocular neovasculogenesis.

NEI Research Program: Retinal and Choroidal Diseases--Diabetic Retinopathy and Other Vascular and Circulatory Abnormalities.

Publications:

BenEzra D: Mediators of immunological reactions: Function as inducers of neovascularisation. Metabolic Ophthalmol (in press).

BenEzra D: Possible mediation of vasculogenesis by products of immune reaction. The Second International Symposium on Immunology and Immunopathology of the Eye. Paris, Masson Co (in press).

BenEzra D: Neovasculogenic ability of prostaglandins growth factors and synthetic chemoattractants. Am J Ophthalmol (in press).



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00071-01 CB
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PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Blepharoconjunctivitis: A Side Effect of 13-cis Retinoic Acid Therapy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Jane Blackman	M.D.	Senior Staff Fellow	CB	NEI
Other:	Gary Peck	M.D.	Senior Investigator	DB	NCI
	Donald Bergsma	M.D.	Senior Staff Ophthalmologist	CB	NEI

COOPERATING UNITS (if any)

Dermatology Branch, NCI  
Microbiology Laboratory, CC

LAB/BRANCH

Clinical Branch

SECTION

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

0.4

PROFESSIONAL:

0.3

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Eighty-four patients with different hyperkeratinizing dermatologic disorders were treated with oral 13-cis retinoic acid (13-cis RA). The 13-cis RA increased the symptoms of blepharoconjunctivitis that were present in 17 patients prior to therapy, and blepharoconjunctivitis occurred in 17 other patients. Staph aureus was cultured from the conjunctiva of 23 patients.

Treatment of the eye lids with topical erythromycin ointment enabled the patients to continue high doses of 13-cis RA. No other toxicity of the 13-cis RA was detected.

Project Description:

Objectives: Patients in the oral 13-cis retinoic acid therapy study for hyperkeratinizing diseases developed blepharoconjunctivitis. (See NCI Project No 76-C64-13-cis RA) The cause of this side effect needed to be determined and treated to enable patients to continue in that study.

Methods Employed: Symptomatic patients and some nonsymptomatic patients were examined by an ophthalmologist. Repeated psychophysical tests were performed on five patients to rule out retinal toxicity. Repeat cultures of lids and conjunctiva and conjunctival scrapings were performed.

Major Findings: Blepharoconjunctivitis developed in 34 of 84 patients treated with oral 13-cis RA for varied dermatologic disorders. Half the patients had blepharoconjunctivitis prior to treatment with 13-cis RA and worsened on therapy. The symptoms began four days to two months after beginning therapy. The severity of blepharoconjunctivitis was dose-related and was seen more frequently in patients with Darier's disease (six of nine patients) or multiple basal cell carcinomas (ten of eleven patients).

Staph aureus was determined by culture to be the causative organism in 23 patients. No particular phage type of staph was responsible for the infections. Therapy with topical erythromycin to the lids was effective in these patients. The patients then could tolerate higher and continued doses of 13-cis RA. The blepharoconjunctivitis of all patients spontaneously cleared within one week of discontinuing 13-cis RA. Electroretinograms and psychophysical testing showed no retinal toxicity associated with the medication as used. (See NCI Project No. 76-C64-13-cis RA)

Significance to Biomedical Research and the Program of the Institute: 13-cis RA is a helpful medication for patients with varied hyperkeratinizing disorders. The bothersome side effect of blepharoconjunctivitis can be managed with topical antibiotics to the extent that patients can continue their dermatologic therapy.

Proposed Course: No further investigation is planned at this point.

NEI Research Program: Corneal Diseases--External Ocular Infections and Inflammatory Diseases

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00074-01 CB																								
PERIOD COVERED October 1, 1977 to September 30, 1978																										
TITLE OF PROJECT (80 characters or less)  Ocular Manifestations of Hyperkeratosis Follicularis																										
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <table style="width: 100%; border: none;"> <tr> <td style="width: 30%;">PI:</td> <td style="width: 30%;">Jane Blackman</td> <td style="width: 10%;">M.D.</td> <td style="width: 25%;">Senior Staff Fellow</td> <td style="width: 10%;">CB</td> <td style="width: 10%;">NEI</td> </tr> <tr> <td>Other:</td> <td>Gary Peck</td> <td>M.D.</td> <td>Senior Investigator</td> <td>DB</td> <td>NCI</td> </tr> <tr> <td></td> <td>Thomas Olson</td> <td>M.D.</td> <td>Clinical Associate</td> <td>DB</td> <td>NCI</td> </tr> <tr> <td></td> <td>Howard Bartner</td> <td>M.A.</td> <td>Chief, Medical Illustration Section</td> <td>MAP</td> <td>DRS</td> </tr> </table>			PI:	Jane Blackman	M.D.	Senior Staff Fellow	CB	NEI	Other:	Gary Peck	M.D.	Senior Investigator	DB	NCI		Thomas Olson	M.D.	Clinical Associate	DB	NCI		Howard Bartner	M.A.	Chief, Medical Illustration Section	MAP	DRS
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	Thomas Olson	M.D.	Clinical Associate	DB	NCI																					
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COOPERATING UNITS (if any)  Dermatology Branch, NCI Medical Illustration Section, MAP, DRS																										
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SECTION																										
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20014																										
TOTAL MANYEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0.0																								
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																										
SUMMARY OF WORK (200 words or less - underline keywords)  Patients with <u>Darier's Disease</u> , <u>hyperkeratosis follicularis</u> , have been said to have involvement of the lid margins and an isolated corneal opacity. We have examined 18 patients and report that 14 have <u>peripheral, deep epithelial, grouped lesions</u> characteristic of this disease. Fourteen of the patients also have <u>central dendritiform irregularity</u> of the <u>corneal surface</u> . Other ocular changes involve the corneal periphery with <u>pannus</u> and <u>marginal ulcers</u> or <u>hyperkeratotic areas on the lid margins</u> .																										

Project Description:

Objectives: The objective of the study was to determine whether the ocular lesions of patients with Darier's disease are affected by retinoic acid therapy or whether these lesions are solely associated with the dermatologic disease. The corneal lesions were observed recurrently to determine the course of the changes.

Methods Employed: Patients with Darier's disease were given complete eye examinations. Drawings and photographs of corneal changes and cultures and conjunctival scrapings were made. Follow-up observation was for 6 to 18 months.

Major Findings: Peripheral grouped, deep epithelial opacities were noted in 14 of 18 patients with Darier's disease, several of whom had not yet begun retinoic acid therapy. Central dendritiform lesions were noted in 14 patients. These were stable and did not change over the period of observation. These changes were not associated with the extent of skin involvement, bacteria present on the eyelids, age of the patient, or any visual problems. The eyelid involvement characterized by hyperkeratotic areas responded similarly to the rest of the skin to treatment with retinoic acid analogs. The corneal changes did not respond to retinoic acid therapy.

Significance to Biomedical Research and the Program of the Institute: The ocular signs of the skin disorder Darier's disease have been documented. Lid lesions improve concurrently with the skin disease during retinoic acid treatment, but the corneal lesions do not change. The ocular signs are benign, usually stationary, and do not threaten vision. The central dendritiform lesions must be added to the list of disorders considered in the differential diagnosis of epithelial keratitis caused by herpes virus infections.

Proposed Course: We plan to study the pathology of the peripheral corneal lesions to elucidate further the pathologic processes of Darier's disease.

NEI Research Program: Corneal Diseases--Corneal Edema, Dystrophies, and Inherited Disorders

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE <b>NOTICE OF</b> INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00058-02 CB																		
PERIOD COVERED October 1, 1977 to September 30, 1978																				
TITLE OF PROJECT (80 characters or less) Suppressor Lymphokine in Mice Induced by Concanavalin A																				
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																				
<table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 30%;">Jane Blackman</td> <td style="width: 10%;">M.D.</td> <td style="width: 30%;">Senior Staff Fellow</td> <td style="width: 10%;">CB</td> <td style="width: 5%;">NEI</td> </tr> <tr> <td>Other:</td> <td>Joost Oppenheim</td> <td>M.D.</td> <td>Chief</td> <td>LMI</td> <td>NIDR</td> </tr> <tr> <td></td> <td>Terrell Hoffeld</td> <td>M.D.</td> <td>Dental Officer</td> <td>LMI</td> <td>NIDR</td> </tr> </table>			PI:	Jane Blackman	M.D.	Senior Staff Fellow	CB	NEI	Other:	Joost Oppenheim	M.D.	Chief	LMI	NIDR		Terrell Hoffeld	M.D.	Dental Officer	LMI	NIDR
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Other:	Joost Oppenheim	M.D.	Chief	LMI	NIDR															
	Terrell Hoffeld	M.D.	Dental Officer	LMI	NIDR															
COOPERATING UNITS (if any) Laboratory of Microbiology and Immunology, NIDR																				
LAB/BRANCH Clinical Branch																				
SECTION																				
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20014																				
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:																		
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SUMMARY OF WORK (200 words or less - underline keywords)																				
<p>Suppressor lymphokines may be important in the regulation of the immune response. We studied in vitro a <u>murine suppressor lymphokine</u> induced by <u>Concanavalin A</u>. This is a nonspecific suppressor which can suppress both a polyclonal <u>blast transformation</u> induced by <u>lipopolysaccharide (LPS)</u> and <u>anti-body synthesis</u> induced by sheep red blood cells (SRBC). Initial steps in purification by <u>sephadex gel filtration</u> showed it to have a molecular weight of 36-72,000 daltons.</p>																				

Project Description:

Objectives: We wished to continue, from the previous year, to evaluate and purify a suppressor lymphokine. The method by which the suppressor lymphokine affects the immune response is unknown. Tadakuma and Pierce have stated ( J Immunol 120:481, 1978) that a nonspecific suppressor, SIRS, acts on macrophages to release a soluble factor which then regulates B cell activity. We wished to investigate if this macrophage factor could be lymphocyte activation factor, a known macrophage product. The long-term objective would be to use this lymphokine to modulate intraocular inflammation, e.g. in uveitis

Methods Employed: Suppressor lymphokine was induced in C57BL/6 mice spleens in vitro with Concanavalin A.

Suppressor activity was assayed in vitro by measuring inhibition of blast transformation initiated by LPS and other B cell mitogens initiated by SRBC in the Jerne plaque system.

Assay of lymphocyte activation factor was performed in vitro with C3H/HeJ mice thymocytes induced with phytohemagglutinin. Gel filtration on Sephadex G100 column was performed to obtain an estimate of molecular weights.

Major Findings: We found that the lymphokine suppressor is a non-specific suppressor of lymphocyte blast transformation and antibody synthesis. The suppressor can be induced and tested in several mouse strains. Both activities of the suppressor lymphokine elute on the G100 column at a molecular weight of 36-72,000 daltons. The suppressor lymphokine does not affect the level of lymphocyte activation factor.

Significance to Biomedical Research and the Program of the Institute: The method by which a suppressor lymphokine called SIRS causes suppression is postulated to be via the release of a soluble macrophage factor. (The suppressor lymphokine we have studied is probably the same as SIRS.) We have not been able to elucidate further the mechanism of action of the suppressor.

Proposed Course: Further purification of the suppressor factor will not be continued.

NEI Research Program: Retinal and Choroidal Diseases--Inflammatory Disorders

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00020-04 CB																														
PERIOD COVERED October 1, 1977 to September 30, 1978																																
TITLE OF PROJECT (80 characters or less)  Parametric Studies of Eye Movement Disorders in Human Beings																																
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 30%;">David G. Cogan</td> <td style="width: 10%;">M.D.</td> <td style="width: 30%;">Medical Officer</td> <td style="width: 10%;">CB</td> <td style="width: 10%;">NEI</td> </tr> <tr> <td>Other:</td> <td>Fred C. Chu</td> <td>M.D.</td> <td>Senior Staff Fellow</td> <td>CB</td> <td>NEI</td> </tr> <tr> <td></td> <td>Douglas B. Reingold</td> <td>M.A.</td> <td>Research Associate</td> <td>CB</td> <td>NEI</td> </tr> <tr> <td></td> <td>Robert D. Reinecke</td> <td>M.D.</td> <td>Guest Worker</td> <td>CB</td> <td>NEI</td> </tr> <tr> <td></td> <td>Dan Milder</td> <td>M.D.</td> <td>Visiting Associate</td> <td>CB</td> <td>NEI</td> </tr> </table>			PI:	David G. Cogan	M.D.	Medical Officer	CB	NEI	Other:	Fred C. Chu	M.D.	Senior Staff Fellow	CB	NEI		Douglas B. Reingold	M.A.	Research Associate	CB	NEI		Robert D. Reinecke	M.D.	Guest Worker	CB	NEI		Dan Milder	M.D.	Visiting Associate	CB	NEI
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COOPERATING UNITS (if any)  Development and Metabolic Neurology Branch, NINCDS																																
LAB/BRANCH Clinical Branch																																
SECTION																																
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SUMMARY OF WORK (200 words or less - underline keywords)  Ocular motor abnormalities are being documented by electro-oculography in patients with neurologic disease. Especially emphasized are the disturbances resulting from cerebellar lesions.																																

Project Description:

Objectives: To improve diagnosis of intracranial disease by characterizing ocular motor disturbances and to learn the role of various portions of the brain, especially the cerebellum, in the control of eye movements.

Methods Employed: Ocular motility is measured by electro-oculography. The patient, equipped with skin electrodes about the eyes, is seated comfortably in a motorized Barany chair and surrounded by a hood that encompasses the entire visual environment. Projected lights on the screen provide the discrete stimuli for saccadic and pursuit movements; projected moving strips provide the stimulus for the optokinetic response. Rotation of the chair elicits the vestibular response. Movements of the eyes are recorded on a 4 channel polygraph and simultaneously monitored by means of an infrared sensitive video camera mounted on the chair in front of the patient. Electro-oculographic data are fed directly into a laboratory computer for analysis.

Major Findings: A substantial data bank has been accumulated representing quantitative information on a variety of ocular motor abnormalities. Cerebellar disturbances have been emphasized leading, among other inferences, to the following generalization. The role of the cerebellum is concerned with the accuracy of saccadic fixation rather than with the velocity of the movement. It is especially important for tracking targets. Pursuit movements appear to be totally dependent on the cerebellum for processing of visual information. Thus, patients with cerebellar lesions show a dysmetria on fixational changes and a replacement of smooth pursuit movements, while tracking a target, by a series of saccades. Among other functions of the cerebellum is its role in prismatic ductions (an entirely new observation).

The surprising conclusion from these and other observations is that the cerebellum processes visual information rather than simply modulating the motor control of the eyes. This is surprising because the cerebellum is generally regarded as a motor organ.

Present studies are being extended to the interrelationships of the vestibular and optokinetic responses in normal persons and in persons with cerebellar deficits.

Aside from the cerebellar studies, observations have been made by video ophthalmoscopy on patients with congenital nystagmus to determine what portion of the retina was used for fixation. The taped recordings are presently being analyzed. Observations have also been made in collaboration with the Development and Metabolic Neurology Branch, National Institute of Neurological and Communicative Disorders and Stroke on ocular motor disturbances in patients with a variety of genetic and metabolic abnormalities. Some of these will be reported in the near future.

Significance to Biomedical Research and the Program of the Institute: Elucidation of the role of the cerebellum has lagged behind that of other major portions of the brain. We have an unusual opportunity in the case of eye

movements to make precise measurements of function, and it is hoped that, when a sufficient number of patients with cerebellar deficits have been tested, it will be possible not only to indicate the role of the cerebellum for at least one function but to determine the topographic localization of this function.

Proposed Course: The project will be continued.

NEI Research Program: Sensory and Motor Disorders of Vision--Sensory and Motor Disorders Related to Specific Disease Processes

Publications: None



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 000154-05 CB
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less)  Experimental Glaucoma in the Rhesus Monkey		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Other:	Douglas E. Gaasterland Teruo Tanishima Helen MacLellan Toichiro Kuwabara  Jonathan Pederson	M.D. Senior Staff Ophthalmologist M.D. Visiting Scientist M.S. Biologist M.D. Head, Section on Experimental Pathology M.D. Clinical Associate
		CB NEI LVR NEI CB NEI  LVR NEI CB NEI
COOPERATING UNITS (if any)  None		
LAB/BRANCH Clinical Branch		
SECTION		
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20014		
TOTAL MANYEARS: 0.4	PROFESSIONAL: 0.3	OTHER: 0.1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The purpose of this investigation is to study the <u>morphology</u> , the <u>physiologic function</u> , and the <u>pharmacologic behavior</u> of the eye of the rhesus monkey in its <u>normal state</u> compared to its state when <u>experimental glaucoma</u> has been induced by argon laser photocoagulation of the <u>trabecular meshwork</u> . One study of the effect of elevated intraocular pressure on optic nerve and retina by <u>light and electron microscopic examination</u> has been completed. The work with <u>whole-mount retinas</u> continues. This has allowed additional interpretation of <u>axoplasmic blockade</u> in glaucoma and identification of the scleral lamina cribrosa as the site of obstruction of <u>axoplasmic transport</u> during chronic elevation of intraocular pressure.		

Project Description:

Objectives: To study physiologic function, pharmacologic behavior, and morphology of the monkey eye after induction of glaucoma by argon laser photocoagulation of the trabecular meshwork. To compare observations to control normal eyes.

Methods Employed: Circumferential argon laser photocoagulation of the rhesus monkey trabecular meshwork eventually causes sustained elevation of intraocular pressure to the range of 30 to 55 mmHg, the pressure range found in many patients with open-angle glaucoma. This is in contrast to the acute short duration, very high pressure elevation (more than 65 mmHg, up to 95 mmHg) seen in most models for glaucoma. Outflow facility is evaluated by perfusion. Aqueous flow is determined by turnover of radioiodinated serum albumin injected into the anterior chamber. Retinal and optic nerve function can be studied by autoradiography and electron microscopy to evaluate morphologic evidence of altered axoplasmic flow. The retina can also be studied in cross section or by preparing whole-mounts of the tissue. Additional studies of the effect of less than circumferential argon laser photocoagulation have been started.

Major Findings: In FY 1978, morphologic study of cross sections of the optic nerve and retina led to the identification of the scleral lamina cribrosa as the site of the blockade of axoplasmic transport in chronic elevation of intraocular pressure. For this study, ten eyes of five monkeys were examined; seven of these eyes had previously induced experimental glaucoma of from 19 to more than 70 days in duration. There has been a six-month hiatus in these studies because the argon laser is not working. As soon as repairs to the laser are completed, these studies will be resumed.

A study has been done in two monkeys with three treated eyes showing the development of optic nerve head cupping by serial fundus photography.

The technique for mounting and examining whole-mounts of monkey retinas is still being learned, and tissue is being collected for this.

Significance to Biomedical Research and the Program of the Institute: All parts of the project are immediately related to clinical problems in glaucoma. This experimental glaucoma is the best model available for human chronic open-angle ("simple") glaucoma. Using this model allows close examination of the retina and optic nerve with the promise of additional insight into the mechanism of loss of visual function in the patient with glaucoma.

Proposed Course: The project will continue.

NEI Research Program: Glaucoma---Etiology of Glaucoma (Primary Open-Angle Glaucoma/Secondary Glaucomas)

Publications:

Gaasterland DE, Tanishima T, Kuwabara T: Axoplasmic flow during chronic experimental glaucoma. I. Light and electron microscopic studies of the monkey optic nerve head during development of glaucomatous cupping. Invest Ophthalmol Visual Sci (in press).



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00046-02 CB												
PERIOD COVERED October 1, 1977 to September 30, 1978														
TITLE OF PROJECT (80 characters or less) Laboratory Studies of Aqueous Humor Dynamics														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT														
<table style="width: 100%; border: none;"> <tr> <td style="width: 40%;">PI: Douglas E. Gaasterland</td> <td style="width: 20%;">M.D.</td> <td style="width: 30%;">Senior Staff Ophthalmologist</td> <td style="width: 10%;">CB NEI</td> </tr> <tr> <td>Other: Jonathan E. Pederson</td> <td>M.D.</td> <td>Clinical Associate</td> <td>CB NEI</td> </tr> <tr> <td>Helen M. MacLellan</td> <td>M.S.</td> <td>Biologist</td> <td>CB NEI</td> </tr> </table>			PI: Douglas E. Gaasterland	M.D.	Senior Staff Ophthalmologist	CB NEI	Other: Jonathan E. Pederson	M.D.	Clinical Associate	CB NEI	Helen M. MacLellan	M.S.	Biologist	CB NEI
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COOPERATING UNITS (if any) None														
LAB/BRANCH Clinical Branch														
SECTION														
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20014														
TOTAL MANYEARS: 1.1	PROFESSIONAL: 0.6	OTHER: 0.5												
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS														
SUMMARY OF WORK (200 words or less - underline keywords) <p>             A number of projects have been ongoing with the aim of investigating <u>intra-ocular fluid movement</u> in rhesus monkeys. A model for <u>ocular hypotony</u> has been devised, using injection of osmotically active materials either between the choroid and sclera or between the retina and choroid producing <u>cilio-choroidal</u> or <u>retinal detachment</u>. Studies of the model including preliminary studies of the effect of medications have been initiated. Studies of the accuracy of noninvasive determination of <u>episcleral venous pressure</u> have been carried out. Pressure determined by cannulation has been compared to non-invasive pressure from a chamber. The pressure from the chamber is too high. Studies of the <u>site of formation</u> of aqueous during hypotony have been completed. After paracentesis, fluid enters the eye from the ciliary processes and from the anterior chamber angle. Based upon previous studies of <u>composition</u> of rhesus <u>aqueous humor</u>, a <u>substitute aqueous humor</u> has been produced and is being tested.           </p>														

Project Description:

Objectives: This project is designed to examine the physiology of intra-ocular fluid movement under varied experimental conditions. The major emphasis is on conventional and secondary outflow mechanisms.

Methods Employed: Various methods of perfusion and cannulation of the eye with subsequent measurement of pressures, flows, and concentrations of various substances were performed.

Major Findings: For various parts of this study either Dr. Gaasterland or Dr. Pederson has been the principal investigator. Hypotony follows subchoroidal or subretinal injection of osmotically active agents. Tested were autologous serum and glutathione-bicarbonate Ringer's solution. By contrast, neither sham operation nor subchoroidal or subretinal injection of silicone oil causes significant alteration of intraocular pressure. The hypotony of this model is of relatively short duration, lasting up to about three weeks. At the maximum point of hypotony, which occurs at two days, aqueous flow is not reduced in eyes with ciliochoroidal detachment. In contrast, eyes with retinal detachment have a lower aqueous flow. Medication studies have been started to try to obtain information on the role of uveal scleral outflow routes in this phenomenon. (Dr. Pederson)

In a group of monkeys, episcleral venous pressure has been measured directly, by cannulation, and indirectly with the pressure chamber. Depending upon the end point chosen, the pressure indicated by the chamber method exceeds the cannulated pressure by a small to a larger amount. This result is relevant to the observation that aqueous flow rate determined by fluorometry is far in excess of that calculated from the Goldmann equation. (Dr. Gaasterland)

In a group of monkeys, some of which were aniridic, hypotony was induced by paracentesis. Photographs were made of the ciliary process and/or the anterior chamber angle following intravenous fluorescein administration. Following paracentesis-induced hypotony, eyes were obtained from other animals for scanning electron microscopy. After paracentesis, fluorescein enters the posterior chamber from the tips of the ciliary processes in isolated locations. Fluorescein enters the anterior chamber angle diffusely, either by a backflow from the canal of Schlemm, or through the root of the iris, or both. Proteins similarly leak into the posterior chamber from the ciliary processes and seem to enter the anterior chamber by backflow through the canal of Schlemm. We had previously observed that after a slow reduction of intra-ocular pressure, the trabecular meshwork functioned as a one-way valve and did not allow backflow into the anterior chamber. The present observations emphasize the importance of the speed of development of hypotony in the subsequent behavior of the altered tissue. (Dr. Pederson)

A formulation for an artificial aqueous humor has been completed based upon monkey aqueous analysis. The solution has been produced in the Media Production Unit of the NIH. In a series of anterior chamber perfusion experi-

ments using this solution in normal monkey eyes, outflow facility was observed to increase no more during a one and one-half hour experiment than previously observed when similar monkey eyes were perfused with pooled rhesus monkey aqueous humor. In a separate series of experiments, the effects of prolonged perfusion with various salt solutions upon the corneal endothelium are being assessed. One method is to look at the corneal epithelium after vital staining with trypan blue. Preliminary results indicate that the substitute aqueous humor causes less dropout of corneal endothelial cells than perfusion with various other salt solutions, and the changes observed resemble those seen in control eyes which have not been perfused. (Dr. Gaasterland)

Significance to Biomedical Research and the Program of the Institute:

The studies are elucidating normal dynamics of aqueous humor, as well as abnormal dynamics in experimentally induced situations, mimicking clinical problems. It is hoped that these studies will yield information applicable to understanding and treating glaucoma and hypotony.

Proposed Course: Similar studies will be continued.

NEI Research Program: Glaucoma--Hydrodynamics of the Eye

Publications:

Pederson JE, Gaasterland DE, MacLellan HM: Experimental ciliochoroidal detachment. Effect on intraocular pressure and aqueous flow. Arch Ophthalmol (in press).

Pederson JE, MacLellan HM, Gaasterland DE: The rate of reflux fluid movement into the eye from Schlemm's canal during hypotony in the rhesus monkey. Invest Ophthalmol Visual Sci 17:377, 1978.

Gaasterland DE, Pederson JE, MacLellan HM: Perfusate effects upon resistance to aqueous humor outflow in the rhesus monkey eye. A comparison of glutathione-biocarbonate Ringer's solution to pooled aqueous humor as perfusate. Invest Ophthalmol Visual Sci 17:391, 1978.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00168-03 CB																														
PERIOD COVERED October 1, 1977 to September 30, 1978																																
TITLE OF PROJECT (80 characters or less)  Laser Surgery for Glaucoma																																
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																																
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SUMMARY OF WORK (200 words or less - underline keywords)																																
<p>           The high energy and power that are found in some <u>laser</u> beams offer a tool for noninvasive alteration of anterior intraocular tissue. Specifically, <u>iridotomy</u> and <u>trabeculotomy</u> are possible. This has importance for <u>glaucoma patients</u> because of the potential improvement of surgical outcome and reduced surgical morbidity. The aim of this project is a systematic evaluation of laser effects in <u>simian</u> (rhesus) <u>eyes</u> and the application of promising systems and procedures to human glaucoma eyes under controlled conditions.         </p>																																

Project Description:

Objectives: To develop workable laser systems for anterior segment surgery and to apply these systems to the normal monkey eye. To study the physiological and morphologic effects of laser energy upon monkey eyes. To apply favorable laser systems under controlled conditions to glaucoma in humans.

Methods Employed: In FY 1978, the third year of this project, there has been continued attention to instrumentation development, while simultaneously a number of studies of the effect of Q-switched ruby laser energy application to the iris have been initiated. Tissue studies for morphology and for physiologic function are done with standard methods: perfusion of the anterior chamber to determine outflow facilities; turnover of Risa to determine flow; and gross, light, and electron microscopic tissue examination.

Major Findings: With this instrument, it is easy to create a through and through hole in the iris. The energy density necessary to create this has been studied. A pulse of light containing approximately 50 millijoules in a spot-size of 100 micro diameter causes a through and through hole in the monkey iris. Higher energies are associated with corneal epithelial and endothelial damage, which is transient. Energies in the range of 300 millijoules are associated with posterior synechia and cataract development. Seven monkeys have had bilateral perfusion of the anterior chamber to determine total facility. These monkeys have now healed and, as soon as the glass gonioscopy lenses become available, will receive monocular treatment of the trabecular meshwork.

Significance to Biomedical Research and the Program of the Institute: Potentially, a physically-noninvasive laser system for anterior segment surgery might replace conventional invasive operative procedures for several types of glaucoma. This possibility is in its infancy at this time.

Proposed Course: The project will be continued.

NEI Research Program: Glaucoma--Etiology of Glaucoma (Primary Open-Angle Glaucoma/Primary Angle-Closure Glaucoma/Secondary Glaucomas)

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00143-05 CB																		
PERIOD COVERED October 1, 1977 to September 30, 1978																				
TITLE OF PROJECT (80 characters or less)  Radioiodinated Chloroquine Analog for Diagnosis of Ocular Melanoma																				
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">Douglas E. Gaasterland</td> <td style="width: 10%;">M.D.</td> <td style="width: 30%;">Senior Staff Ophthalmologist</td> <td style="width: 5%;">CB</td> <td style="width: 5%;">NEI</td> </tr> <tr> <td>Other:</td> <td>Elmer J. Ballintine</td> <td>M.D.</td> <td>Clinical Director</td> <td>CB</td> <td>NEI</td> </tr> <tr> <td></td> <td>Carl Kupfer</td> <td>M.D.</td> <td>Director</td> <td></td> <td>NEI</td> </tr> </table>			PI:	Douglas E. Gaasterland	M.D.	Senior Staff Ophthalmologist	CB	NEI	Other:	Elmer J. Ballintine	M.D.	Clinical Director	CB	NEI		Carl Kupfer	M.D.	Director		NEI
PI:	Douglas E. Gaasterland	M.D.	Senior Staff Ophthalmologist	CB	NEI															
Other:	Elmer J. Ballintine	M.D.	Clinical Director	CB	NEI															
	Carl Kupfer	M.D.	Director		NEI															
COOPERATING UNITS (if any)  None																				
LAB/BRANCH Clinical Branch																				
SECTION																				
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20014																				
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:																		
0.1	0.1	0.0																		
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																				
SUMMARY OF WORK (200 words or less - underline keywords)  The purpose of this clinical investigation was to assess the value of system- ically administered <u>I-125 labelled chloroquine analog</u> for the <u>detection of</u> <u>ocular melanoma</u> . Patient enrollment terminated 30 June 75, after the 36th patient was accepted. Current interest is in continued follow-up examination of the patients. This will yield information regarding the clinical course of diagnosed and treated melanoma patients, of diagnosed melanoma patients who refused treatment, and of patients with lesions which may or may not be ocular melanoma. The course will be compared to the results of the <u>radioac-</u> <u>tive tracer testing</u> .																				

Project Description:

Objectives: To determine the value of using I-125 labelled chloroquine analog for the detection of ocular melanoma.

Methods Employed: During this year a number of standard follow-up clinical examinations have been performed.

Major Findings: A number of the original group of 36 patients continue to be seen intermittently or to correspond with the study's investigators giving information concerning their clinical course. One patient who had a diagnosis of malignant melanoma in 1974, and has not previously been mentioned in NEI Annual Reports, had an enucleation at that time. She wrote a month ago to report that she has developed metastatic disease in her liver, bone marrow, and skin. She is undergoing systemic chemotherapy in Tennessee. The two patients who refused enucleation in 1973 and 1974 continue under observation, still refusing enucleation. Both these tumors have been documented to grow. The patient with the positive test in 1974, but whose lesion had been under observation for 15 years, continues to be seen here periodically with no change in the lesion.

Significance to Biomedical Research and the Program of the Institute: Continued follow-up information concerning the course of the enucleated patients and the other patients is important. The registry of melanoma patients created by this project serves as a resource.

Proposed Course: The intermittent examinations of this small group of patients will be continued.

NEI Research Program: Retinal and Choroidal Diseases--Tumors

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00030-07 CB																																										
PERIOD COVERED October 1, 1977 to September 30, 1978																																												
TITLE OF PROJECT (80 characters or less)  Studies of Parameters of Intraocular Pressure																																												
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	Eric Linner	M.D.	Visiting Scientist	CB	NEI																																							
COOPERATING UNITS (if any) Normal Volunteer Office, CC, NIH; Pharmaceutical Development Service, NIH; Biomedical and Engineering Instrumentation Branch, DRS, NIH																																												
LAB/BRANCH Clinical Branch																																												
SECTION																																												
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20014																																												
TOTAL MANYEARS: 1.25	PROFESSIONAL: 0.5	OTHER: 0.75																																										
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																																												
SUMMARY OF WORK (200 words or less - underline keywords)  In this continuing study of the <u>parameters of intraocular pressure</u> , young and old <u>normal volunteers</u> and patients with <u>glaucoma</u> and <u>ocular hypertension</u> participate. There is interest in determining the actual values of the parameters in eyes not affected by medications and in determining the acute and chronic <u>effects of antiglaucoma medications</u> alone and in combination upon the parameters in normal and in diseased eyes.																																												

Project Description:

Objectives: To evaluate parameters of intraocular pressure in normal eyes and eyes with ocular hypertension or glaucoma before and after anti-glaucoma medications.

Methods Employed: Replicate studies are done upon sophisticated human participants. Seven parameters are determined before and after medication: intraocular pressure, episcleral venous pressure, total facility, true facility of outflow, pseudofacility, aqueous flow, and ocular rigidity. The  $P_K$  of Goldmann is no longer determined because it does not add any more information. Acute drug effects are emphasized. Chronic drug effects are studied by use of the Ocusert system (Alza Laboratories) for pilocarpine and in patients receiving monocular treatment in the ocular hypertension protocol of Dr. Ballentine (Z01 00150-05 CB).

Major Findings: During his sabbatical from Gothenburg, Dr. Eric Linnér has been able to join our staff to study ocular hypertensive patients. A group of patients was recruited, and evaluation in the laboratory has shown that these patients, who do not have glaucomatous visual field defects have a rate of calculated flow of aqueous humor far in excess of that observed in normal volunteers of the same age. The responsiveness of the intraocular pressure and the aqueous flow of these patients to an oral dose of 500 mg of acetazolamide has been assessed. The majority of these patients show a small response, but several show a large magnitude response. These latter patients resemble the glaucoma patients previously studied in Sweden. The patterns of correlations of the observations are being studied.

A study of the reproducibility of tonographic results and of whether the application of the neck cuff for pseudofacility studies affects the study of tonography has been initiated. Seven patients have been studied and more are being recruited.

Significance to Biomedical Research and the Program of the Institute: Study of patterns of alteration in the parameters of intraocular pressure by glaucoma medications allows clearer understanding of their mechanisms of action. Studies of these parameters more clearly define the difference between normal and abnormal. The measurements can be extrapolated to more basic physiologic functions, yielding insight to the function of the human eye. This information is unique in ophthalmic research.

Proposed Course: The project will be continued.

NEI Research Program: Glaucoma--Etiology of Glaucoma (Primary Open-Angle Glaucoma/Secondary Glaucomas)

Publications:

Gaasterland DE, Kupfer C, Milton R, Ross K, McCain L, MacLellan H: Studies of aqueous humor dynamics in man. VI. Effects of age upon parameters of intraocular pressure in normal human eyes. Exp Eye Res (in press).

## PERIOD COVERED

October 1, 1977 to September 30, 1978

## TITLE OF PROJECT (80 characters or less)

Treatment of Neovascular Glaucoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER  
PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Douglas E. Gaasterland	M.D.	Senior Staff Ophthalmologist	CB	NEI
Other:	Elmer J. Ballintine	M.D.	Clinical Director	CB	NEI
	Jonathan E. Pederson	M.D.	Clinical Associate	CB	NEI
	Helen MacLellan	M.S.	Biologist	CB	NEI
	Richard Stone	M.D.	Clinical Associate	CB	NEI

## COOPERATING UNITS (if any)

None

## LAB/BRANCH

Clinical Branch

## SECTION

## INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20014

## TOTAL MANYEARS:

0.2

## PROFESSIONAL:

0.2

## OTHER:

0.0

## CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS      ☐ (b) HUMAN TISSUES      ☐ (c) NEITHER

☐ (a1) MINORS    ☐ (a2) INTERVIEWS

## SUMMARY OF WORK (200 words or less - underline keywords)

Patients with rubeosis iridis and neovascular glaucoma are being recruited. Those with salvageable vision are invited to join this prospective, randomized study of whether cyclocryotherapy or cyclodiathermy is better for the treatment of this disease. The protocol for this study has been written and initial patients recruited. Outcome will be judged by assessing preservation of visual function; adequate control of intraocular pressure, with or without medications; and control of discomfort. It is estimated that approximately 40 nondiabetic and 40 diabetic patients will be needed to give answers in this project.

Project Description:

Objectives: To determine whether one of two methods for ciliary body ablation, cyclodiathermy or cyclocryotherapy, is better for treatment of neovascular glaucoma.

Methods Employed: Patients who are eligible to join the study, and who give informed consent to join, are randomly assigned to receive one of the two methods of treatment. Follow-up is aimed at identifying adequacy of treatment and complications.

Major Findings: None

Significance to Biomedical Research and the Program of the Institute: This study will give clarification of the proper management of these difficult secondary glaucoma patients.

Proposed Course: The study will be continued.

NEI Research Program: Glaucoma--Medical and Surgical Treatment of Glaucoma

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00006-07 CB																		
PERIOD COVERED <p style="text-align: center;">October 1, 1977 to September 30, 1978</p>																				
TITLE OF PROJECT (80 characters or less)  <p style="text-align: center;">Research in Methods of Evaluating Visual Processes</p>																				
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 25%;">Ralph D. Gunkel</td> <td style="width: 10%;">O.D.</td> <td style="width: 30%;">Ophthalmic Physicist</td> <td style="width: 10%;">CB</td> <td style="width: 10%;">NEI</td> </tr> <tr> <td>Other:</td> <td>David G. Cogan</td> <td>M.D.</td> <td>Medical Officer</td> <td>CB</td> <td>NEI</td> </tr> <tr> <td></td> <td>Fred C. Chu</td> <td>M.D.</td> <td>Senior Staff Fellow</td> <td>CB</td> <td>NEI</td> </tr> </table>			PI:	Ralph D. Gunkel	O.D.	Ophthalmic Physicist	CB	NEI	Other:	David G. Cogan	M.D.	Medical Officer	CB	NEI		Fred C. Chu	M.D.	Senior Staff Fellow	CB	NEI
PI:	Ralph D. Gunkel	O.D.	Ophthalmic Physicist	CB	NEI															
Other:	David G. Cogan	M.D.	Medical Officer	CB	NEI															
	Fred C. Chu	M.D.	Senior Staff Fellow	CB	NEI															
COOPERATING UNITS (if any)  <p style="text-align: center;">None</p>																				
LAB/BRANCH <p style="text-align: center;">Clinical Branch</p>																				
SECTION  																				
INSTITUTE AND LOCATION <p style="text-align: center;">National Eye Institute, NIH, Bethesda, Maryland 20014</p>																				
TOTAL MANYEARS: <p style="text-align: center;">1.5</p>	PROFESSIONAL: <p style="text-align: center;">1.5</p>	OTHER: <p style="text-align: center;">0.0</p>																		
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <span><input checked="" type="checkbox"/> (a) HUMAN SUBJECTS</span> <span><input type="checkbox"/> (b) HUMAN TISSUES</span> <span><input type="checkbox"/> (c) NEITHER</span> </div> <div style="display: flex; justify-content: space-between; margin-top: 5px;"> <span><input type="checkbox"/> (a1) MINORS</span> <span><input type="checkbox"/> (a2) INTERVIEWS</span> </div>																				
SUMMARY OF WORK (200 words or less - underline keywords)  <p>The general purpose and intent of this project is to conduct tests, research, and experiments directed toward the use, improvement, and development of clinical procedures and instruments for measuring functions or properties relating to vision and the eyes. This has consisted primarily of <u>subjective measurements</u> of <u>visibility</u> and <u>chromaticity thresholds</u>. Electroretinography, which is an <u>objective measurement</u> of <u>electrophysiological activity</u> in the retina, is used only in selected cases because of its limited usefulness.</p> <p>The most significant work of the year was the measurement and analysis of defects in color vision with the <u>chromagraph</u>. This is our new instrument which permits the measurement of any type of color defect, many of which are too subtle or too bizarre to be shown with the conventional tests.</p>																				

Project Description:

Objectives: To discover and utilize the most effective and least traumatic methods for quantitating and evaluating any changes in the eye or its adnexae brought about by disease conditions, toxic materials, or degenerative processes. Objective methods are vigorously sought but are not often attainable. The goal of this project continues to be to provide information which will contribute toward the maintenance or restoration of normal visual function wherever possible.

Methods Employed: Commercially available instruments and those developed here are used in measuring rod and cone thresholds, chromaticity thresholds, and other ocular functions in clinic patients. There is frequent consultation with clinical associates and staff members regarding test methods and results, applicability, interpretation, new ideas, and properties of materials.

Major Findings: Psychophysical tests were done on 495 subjects for the purpose of diagnosing or evaluating toxic, inflammatory, degenerative, or congenital retinal conditions.

Various small optical, electrical, and mechanical devices were designed and/or constructed for use in the projects of other staff members.

The chromagraph has been confirmed as giving more comprehensible information about color vision than all of the conventional tests combined. The test is carried out quickly and the results are simultaneously recorded on a chromaticity diagram which is so simple as to be understood at a glance. Many color defects have been plotted in subjects who were classed as normal by all of the other test methods.

Present efforts are directed towards enlarging the file of color defects and correlating specific defects with specific disease entities. Obviously, a considerable number of cases will be required to validate such a study.

Significance to Biomedical Research and the Program of the Institute: Data and information obtained in psychophysical tests contribute materially to NEI's clinical research program.

Associating certain defects in color vision, however subtle, with known pathologies has had very limited success heretofore, but the chromagraph appears to be providing a means to that end.

Proposed Course: The project will be continued.

NEI Research Program: Retinal and Choroidal Diseases--Retinal Organization and Visual Adaptation

Publications:

Gunkel RD, Cogan DG: Colorimetry by a new principle. Arch Ophthalmol  
96:331-334, 1978.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00082-01 CB
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PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Characterizations of Proteoglycans of the Corneal Stroma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	John Hassell	Ph.D.	Staff Fellow	CB	NEI
Other:	David A. Newsome	M.D.	Senior Staff Ophthalmologist	CB	NEI

COOPERATING UNITS (if any)

Cornea Department, Eye Research Institute of Retina Foundation

LAB/BRANCH

Clinical Branch

SECTION

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

0.75

PROFESSIONAL:

0.75

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS      ☒ (b) HUMAN TISSUES      ☐ (c) NEITHER

☐ (a1) MINORS    ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Of the two major classes of structural macromolecules in the corneal stroma, the proteoglycans are less well described. However, both collagen and proteoglycans are crucial to the development and maintenance of optical clarity of the cornea. Two major proteoglycans synthesized by rhesus monkey corneas in culture have been characterized and seem to be similar to those synthesized under the same conditions by human material. Alterations in the normal composition of proteoglycans may accompany or even be the mechanism for certain visually disabling corneal diseases.

Project Description:

Objectives: The extracellular matrix of the human corneal stroma consists of a network of collagen fibers and proteoglycans. The orderly interaction and arrangement of these structural macromolecules is crucial to the transparency of the cornea. Although much is known about the orientation, distribution, and types of collagen present in the stroma, little is known of the nature of the proteoglycans. The purpose of this study is to characterize the proteoglycans present in the stroma of normal and abnormal diseased human corneas.

Methods Employed: Corneas from a variety of species including man were radioactively labeled in organ culture using  $^{35}\text{S}$ -Sulfate and  $^3\text{H}$ -glucosamine. Cleanly dissected stromas were extracted with guanidinium hydrochloride, and the extracted proteoglycans were fractionated by molecular sieve chromatography. The polysaccharide side chains were isolated and analyzed for disaccharide types.

Major Findings: Two major proteoglycans have been isolated from rhesus monkey corneal stroma. The most abundant of these proteoglycans consists of a protein backbone with one to two chondroitin sulfate side chains of 60,000 molecular weight and one or more glycoprotein type polysaccharide side chains of 2,500 molecular weight. The other proteoglycan contains keratan sulfate. The pattern of human stromal proteoglycans appears to be quite similar to that worked out for primate material.

Significance to Biomedical Research and the Program of the Institute: Many diseases result in an opaque and therefore visually disabling cornea. The biochemical basis for corneal opacity is incompletely understood. This study should contribute important knowledge about the role of the stromal proteoglycans in the development and maintenance of corneal transparency and that will aid in the devising of nonsurgical therapies for the prevention of corneal opacity or restoration of optical clarity.

Proposed Course: The composition of corneal stromal proteoglycans synthesized during corneal development, during scarring and wound repair, and during the development of certain corneal dystrophies will be determined in human material.

NEI Research Program: Corneal Diseases--Corneal Transplantation and Stromal Injury and Repair/Corneal Edema, Dystrophies, and Inherited Disorders

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE <b>NOTICE OF</b> INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00079-01 CB						
PERIOD COVERED October 1, 1977 to September 30, 1978								
TITLE OF PROJECT (80 characters or less)  Mechanism of Action of Vitamin A on Corneal Epithelium								
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: John R. Hassell</td> <td style="width: 33%;">Ph.D. Staff Fellow</td> <td style="width: 34%; text-align: right;">CB NEI</td> </tr> <tr> <td>Other: David A. Newsome</td> <td>M.D. Senior Staff Ophthalmologist</td> <td style="text-align: right;">CB NEI</td> </tr> </table>			PI: John R. Hassell	Ph.D. Staff Fellow	CB NEI	Other: David A. Newsome	M.D. Senior Staff Ophthalmologist	CB NEI
PI: John R. Hassell	Ph.D. Staff Fellow	CB NEI						
Other: David A. Newsome	M.D. Senior Staff Ophthalmologist	CB NEI						
COOPERATING UNITS (if any) None								
LAB/BRANCH Clinical Branch								
SECTION								
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20014								
TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0.0						
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS								
SUMMARY OF WORK (200 words or less - underline keywords)  Experiments are being conducted on whole <u>corneas</u> obtained from normal and <u>vitamin A deficient animals</u> . The corneas were radioactively labeled in <u>organ culture</u> and the <u>epithelial glycoproteins</u> were separated by gel electrophoresis. <sup>3</sup> H glucosamine labeling shows that repleting vitamin A deficient rats with <u>retinoic acid</u> restores the <u>synthesis</u> of three major <u>glycoconjugates</u> to normal levels within 24 hours. Repletion with excessive levels of retinoic acid further stimulates the synthesis of these glycoconjugates by the corneal epithelium.								

Project Description:

Objectives: Although vitamin A has been shown to inhibit keratinization of corneal and various other epithelia, the mechanism by which vitamin A acts to maintain a normal epithelium is not well understood. The purpose of this study is to determine the biochemical basis for the vitamin A mediated changes in corneal epithelium.

Methods Employed: Corneas from normal and vitamin A deficient animals were excised and radioactively labeled in organ culture. The epithelium was then harvested, the epithelial proteins separated by gel electrophoresis, and the gel analyzed for radioactivity using autoradiography.

Major Findings:  $^3\text{H}$  glucosamine labeling shows that repleting vitamin A deficient rats with retinoic acid restores the synthesis of three major glycoconjugates to normal levels within 24 hours. Repletion with excessive levels of retinoic acid further stimulated the synthesis of these glycoconjugates. These results suggest that vitamin A regulates the synthesis of these three major glycoconjugates.  $^{14}\text{C}$  Leucine labeling of the epithelial protein was unchanged. This would suggest that in affecting epithelial differentiation, vitamin A alters glycosylation rather than altering protein synthesis.

Significance to Biomedical Research and the Program of the Institute: Xerophthalmia, which can progress to keratomalacia, is a human corneal disease that is thought to arise, in part, from vitamin A deficiency. This disease involves the keratinization of the corneal epithelium and can lead to blindness. The knowledge gained from this study will indicate the biochemical processes of epithelial differentiation that are directly regulated by vitamin A and thereby permit more effective use of vitamin A as a therapeutic agent. Furthermore, this approach will allow the development of diagnostic procedures that will be useful in clinically evaluating human epithelial diseases.

Proposed Course: The functional role of these glycoconjugates in epithelial differentiation and the mechanism by which vitamin A regulates its synthesis will be determined. The glycoconjugates will be isolated and purified by using molecular sieve and affinity chromatography. The amino acid and carbohydrate composition of these purified glycoconjugates will be determined. Antibodies will be made against the glycoconjugates and will be used in determining the cellular localization as well as its sites and rates of synthesis. The antibody will also be very useful in the development of clinical procedures for diagnosis of human corneal epithelial disorders.

NEI Research Program: Corneal Diseases--Dry Eyes and Tear Abnormalities, Epithelial Disorders, and Drug Delivery

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00085-01 CB																																
PERIOD COVERED October 1, 1977 to September 30, 1978																																		
TITLE OF PROJECT (80 characters or less)  The HLA and ABO Antigens and Immunologic Studies in Cogan's Syndrome																																		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																																		
<table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 30%;">Muriel I. Kaiser-Kupfer</td> <td style="width: 20%;">M.D.</td> <td style="width: 30%;">Senior Staff Ophthalmologist</td> <td style="width: 10%;">CB</td> <td style="width: 10%;">NEI</td> </tr> <tr> <td rowspan="5">Other:</td> <td>David G. Cogan</td> <td>M.D.</td> <td>Senior Staff Ophthalmologist</td> <td>CB</td> <td>NEI</td> </tr> <tr> <td>Luis del Valle</td> <td>M.D.</td> <td>Staff Fellow</td> <td>CB</td> <td>NEI</td> </tr> <tr> <td>Kamal K. Mittal</td> <td>Ph.D.</td> <td>Research Microbiologist</td> <td>BB</td> <td>FDA</td> </tr> <tr> <td>Barton Haynes</td> <td>M.D.</td> <td>Staff Fellow</td> <td>LCI</td> <td>NIAID</td> </tr> <tr> <td>Anthony Fauci</td> <td>M.D.</td> <td>Senior Physician</td> <td>LCI</td> <td>NIAID</td> </tr> </table>			PI:	Muriel I. Kaiser-Kupfer	M.D.	Senior Staff Ophthalmologist	CB	NEI	Other:	David G. Cogan	M.D.	Senior Staff Ophthalmologist	CB	NEI	Luis del Valle	M.D.	Staff Fellow	CB	NEI	Kamal K. Mittal	Ph.D.	Research Microbiologist	BB	FDA	Barton Haynes	M.D.	Staff Fellow	LCI	NIAID	Anthony Fauci	M.D.	Senior Physician	LCI	NIAID
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	Anthony Fauci	M.D.	Senior Physician	LCI	NIAID																													
COOPERATING UNITS (if any) Bureau of Biologics, Food and Drug Administration Laboratory of Clinical Investigation, National Institute of Allergy and Infectious Diseases																																		
LAB/BRANCH Clinical Branch																																		
SECTION																																		
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20014																																		
TOTAL MANYEARS: 1.0	PROFESSIONAL: 0.7	OTHER: 0.3																																
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SUMMARY OF WORK (200 words or less - underline keywords)  The purpose of this protocol is to determine the <u>phenotype frequency</u> of the <u>HLA and ABO antigens</u> as well as to explore the possibility of <u>altered immune</u> <u>response</u> in patients with <u>Cogan's syndrome</u> .																																		

Project Description:

Objectives: To determine the HLA and ABO antigens in patients with Cogan's syndrome. To determine in vitro immunologic studies on serum, blood, or separated mononuclear cells.

Methods Employed: Patients having Cogan's syndrome are examined according to a standard set of procedures to confirm the diagnosis. Blood specimens are analyzed to HLA and ABO antigens and a prescribed battery of in vitro immunologic studies.

Major Findings: Patients with Cogan's syndrome do not have a specific HLA type.

Significance to Biomedical Research and the Program of the Institute: To determine the immunologic basis of an eye disease.

Proposed Course: Continue follow-up one year.

NEI Research Program: Corneal Diseases--External Ocular Infections and Inflammatory Diseases

Publications:

Kaiser-Kupfer MI, Mittal KK, del Valle LA, Haynes B: The HLA antigens in Cogan's syndrome. Am J Ophthalmol 86:314-316, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00018-04 CB												
PERIOD COVERED October 1, 1978 to September 30, 1978														
TITLE OF PROJECT (80 characters or less)  Ophthalmologic Screening for Metastatic Lesions to the Eye														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 45%;">Muriel I. Kaiser-Kupfer</td> <td style="width: 20%;">M.D.</td> <td style="width: 20%;">Senior Staff Ophthalmologist</td> <td style="width: 10%;">CB</td> <td style="width: 10%;">NEI</td> </tr> <tr> <td>Other:</td> <td>Joan Bull</td> <td>M.D.</td> <td>Senior Staff Fellow</td> <td>CB</td> <td>NCI</td> </tr> </table>			PI:	Muriel I. Kaiser-Kupfer	M.D.	Senior Staff Ophthalmologist	CB	NEI	Other:	Joan Bull	M.D.	Senior Staff Fellow	CB	NCI
PI:	Muriel I. Kaiser-Kupfer	M.D.	Senior Staff Ophthalmologist	CB	NEI									
Other:	Joan Bull	M.D.	Senior Staff Fellow	CB	NCI									
COOPERATING UNITS (if any)  National Cancer Institute														
LAB/BRANCH Clinical Branch														
SECTION														
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20014														
TOTAL MANYEARS: 0.8	PROFESSIONAL: 0.3	OTHER: 0.5												
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS														
SUMMARY OF WORK (200 words or less - underline keywords)  The purpose of this project is to determine the <u>incidence of metastatic eye disease</u> in patients with <u>metastatic breast carcinoma</u> , to evaluate the <u>effects of irradiation on the eye</u> in patients who have metastatic disease and receive irradiation in conjunction with chemotherapy, and to monitor patients receiving chemotherapy for ocular toxicity. The change in size of choroidal lesions during therapy may serve as an indication of a therapeutic response elsewhere in the body.														

Project Description:

Objectives: To determine the incidence of metastatic eye disease in patients with metastatic breast carcinoma, to evaluate the effects of irradiation on ocular tumors which threaten central vision and monitor the effects of irradiation and chemotherapy on the eye, and to evaluate the effectiveness of hormonal manipulations and chemotherapy on ocular metastasis in relation to systemic effects on the tumor.

Methods Employed: All NCI patients having metastatic breast carcinoma are examined ophthalmoscopically. Those patients having metastatic disease to the eye are then followed frequently as indicated. The course of the ocular metastatic disease is followed with serial color and infrared fundus photography, Goldmann perimetry, and fluorescein fundus photos when indicated.

Major Findings: To date, approximately 100 patients have been seen, and, of those, approximately 13 patients have had evidence of ocular metastasis. Several patients have been found to have developed secondary keratitis following radiation therapy to the posterior choroid. Ocular toxicity of Tamoxifen has been discovered.

Significance to Biomedical Research and the Program of the Institute: The response of choroidal metastatic lesions to cancer chemotherapy could serve as an indication of response to metastatic disease elsewhere in the body.

Proposed Course: The project will continue for one additional year.

NEI Research Program: Retinal and Choroidal Diseases--Tumors

Publications:

Kaiser-Kupfer MI, Lippman ME: Tamoxifen retinopathy. Cancer Treat Rep 62:315-320, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00083-01 CB									
PERIOD COVERED - October 1, 1977 to September 30, 1978											
TITLE OF PROJECT (80 characters or less)  The Pathogenesis of Gyrate Atrophy and Trial of Pyridoxine											
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: Muriel I. Kaiser-Kupfer</td> <td style="width: 33%;">M.D. Senior Staff Ophthalmologist</td> <td style="width: 33%;">CB NEI</td> </tr> <tr> <td>Other: Luis DelValle</td> <td>M.D. Visiting Scientist</td> <td>CB NEI</td> </tr> <tr> <td>David Valle</td> <td>M.D. Assistant Professor, The Johns Hopkins School of Medicine</td> <td></td> </tr> </table>			PI: Muriel I. Kaiser-Kupfer	M.D. Senior Staff Ophthalmologist	CB NEI	Other: Luis DelValle	M.D. Visiting Scientist	CB NEI	David Valle	M.D. Assistant Professor, The Johns Hopkins School of Medicine	
PI: Muriel I. Kaiser-Kupfer	M.D. Senior Staff Ophthalmologist	CB NEI									
Other: Luis DelValle	M.D. Visiting Scientist	CB NEI									
David Valle	M.D. Assistant Professor, The Johns Hopkins School of Medicine										
COOPERATING UNITS (if any)  Department of Pediatrics and Medicine, The Johns Hopkins University School of Medicine, Baltimore, Maryland											
LAB/BRANCH Clinical Branch											
SECTION											
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20014											
TOTAL MANYEARS: 1.5	PROFESSIONAL: 0.5	OTHER: 1.0									
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS											
SUMMARY OF WORK (200 words or less - underline keywords)  Patients with <u>gyrate atrophy</u> of the <u>retina</u> are examined systematically to confirm the diagnosis. Lymphocytes from the patient's blood are isolated, transformed in tissue culture, and assayed for <u>ornithine aminotransferase</u> activity. Skin fibroblasts grown in <u>tissue culture</u> are assayed for ornithine aminotransferase. Other enzymatic activities related to ornithine metabolism such as ornithine decarboxylase activity will be measured. The results will be examined for correlation with the presence of homo- or heterozygosity for the disease trait. Patients will be given pyridoxine to see if the serum concentration of ornithine can be reduced, and, if so, the patient will be classified as a "responder", treatment with pyridoxine will be continued. Responder patients will be observed for arrest in the progress of their disease.											

Project Description:

Objectives: To determine the biochemical processes responsible for the elevated serum ornithine and the retinal lesion that occur in gyrate atrophy of the retina. To determine which patients respond to pyridoxine treatment with a decrease in serum ornithine concentration. To determine if treatment of "responders" with pyridoxine will arrest the progress of the retinal atrophy.

Methods Employed: Patients suspected of having gyrate atrophy of the retina are examined according to a standard set of procedures to confirm the diagnosis. Serum ornithine concentration is measured periodically. Lymphocytes from the patient's blood are transformed and assayed for enzymatic activity related to ornithine metabolism. Punch biopsies of the skin are grown in tissue culture, and their enzymatic activity related to ornithine metabolism is measured.

Major Findings: Patients with gyrate atrophy of the retina have been shown to have a deficiency of ornithine aminotransferase.

Significance to Biomedical Research and the Program of the Institute: Gyrate atrophy of the retina is one of the first of the genetically determined isolated severe retinal degenerations for which a specific biochemical concomitant defect has been demonstrated. The study will guide and test the efficacy of treatment for this blinding eye disease and serve as a model for the investigation of other genetically determined retinal degenerations.

Proposed Course: Continue for at least three years.

NEI Research Program: Retinal and Choroidal Diseases--Developmental and Hereditary Disorders

Publications:

Kaiser-Kupfer MI, Valle D, DelValle L: A specific enzyme defect in gyrate atrophy. Am J Ophthalmol 85:200-204, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00011-04 CB												
PERIOD COVERED October 1, 1977 to September 30, 1978														
TITLE OF PROJECT (80 characters or less)  Pigment Dispersion With and Without Glaucoma														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">Muriel I. Kaiser-Kupfer</td> <td style="width: 15%;">M.D.</td> <td style="width: 30%;">Senior Staff Ophthalmologist</td> <td style="width: 5%;">CB</td> <td style="width: 5%;">NEI</td> </tr> <tr> <td>Other:</td> <td>Luis DelValle</td> <td>M.D.</td> <td>Visiting Scientist</td> <td>CB</td> <td>NEI</td> </tr> </table>			PI:	Muriel I. Kaiser-Kupfer	M.D.	Senior Staff Ophthalmologist	CB	NEI	Other:	Luis DelValle	M.D.	Visiting Scientist	CB	NEI
PI:	Muriel I. Kaiser-Kupfer	M.D.	Senior Staff Ophthalmologist	CB	NEI									
Other:	Luis DelValle	M.D.	Visiting Scientist	CB	NEI									
COOPERATING UNITS (if any)  None														
LAB/BRANCH Clinical Branch														
SECTION														
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20014														
TOTAL MANYEARS: 0.9	PROFESSIONAL: 0.7	OTHER: 0.2												
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS														
SUMMARY OF WORK (200 words or less - underline keywords)  The purpose of this project is to compare patients having <u>pigment dispersion syndrome with and without glaucoma</u> . The data acquired may enable a determination of the risk of patients with pigment dispersion syndrome to develop glaucoma as well as add to understanding of the pathology of the disease state.														

Project Description:

Objectives: To compare patients having pigment dispersion with and without glaucoma by documenting and following the clinical features and course of their disease and by evaluating the patient's performance on a variety of diagnostic tests. To determine the presence of abnormal aqueous humor dynamics using provocative testing in those patients having pigmentary dispersion with and without glaucoma. To compare pigment dispersion with and without glaucoma with respect to possible genetic markers (i.e. lymphocyte transformation, HLA and ABO antigens and family history of open-angle glaucoma). To determine whether pupillary responses to light stimulation are abnormal in cases having iris transillumination.

Methods Employed: At the first visit, the following examinations are performed:

- Complete family history with detailed pedigree
- Best corrected visual acuity with manifest refraction
- Slit lamp examination
- Visual field examination (Goldmann I<sub>2e</sub> and I<sub>4e</sub>)
- Applanation Goldmann tension (app)
- Photography of iris transillumination
- Goniophotography
- Blood specimen for HLA and ABO antigen typing

At the next visit, the following examinations are performed:

- Static perimetry
- Base-line tonography and water-drinking tonography one hour later
- Fasting blood sugar when indicated

At the third visit, the following examinations are performed:

- Slit lamp photography of Krukenberg spindle
- Dilated ophthalmoscopic examination (10% phenylephrine and 1% cyclogel)
- Stereophotographs of the optic nervehead

At the fourth visit, pupillography is performed.

Major Findings: Patients may have pigment dispersion syndrome for as long as 20 years without developing glaucoma.

There may be a hereditary predisposition in some cases, as seen in a mother and daughter, two brothers, and a brother and sister.

Steroid testing and PTC taste testing do not appear to show any particular categorization of these patients. Recent evidence has indicated that HLA antigens in patients with pigment dispersion are also not significantly different than those in the normal population.

It may be noted that whether filtering procedures are performed or not, pigment may be lost from the trabecular meshwork in time.

Significance to Biomedical Research and the Program of the Institute:  
These data may enable a determination to be made of the risk of patients having pigment dispersion to develop glaucoma. It may be possible to identify which features of these determinations have predictive value in forecasting which of those patients having pigment dispersion will develop a visual field defect. In addition, the relationship of "pigmentary" glaucoma to the known characteristics of open-angle glaucoma can be investigated.

Proposed Course: This project will be continued for three more years.

NEI Research Program: Glaucoma--Etiology of Glaucoma (Developmental Glaucoma/Secondary Glaucomas)

Publications:

Kaiser-Kupfer MI, Mittal KK: The HLA and ABO antigens in pigment dispersion syndrome. Am J Ophthalmol 85:368-372, 1978.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE <b>NOTICE OF</b> INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00062-02 QB
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less)  Progressive Essential Iris Atrophy		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Muriel I. Kaiser-Kupfer Other: Carl Kupfer Rodney Lynk	M.D. M.D. M.D.	Senior Staff Ophthalmologist   CB   NEI Director                               NEI Medical Officer                       CB   NEI
COOPERATING UNITS (if any)  None		
LAB/BRANCH Clinical Branch		
SECTION		
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20014		
TOTAL MANYEARS: .56	PROFESSIONAL: .44	OTHER: .12
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)  Patients are being recruited with <u>progressive essential iris atrophy</u> with or without associated corneal disease. Information is being gathered to evaluate the clinical features and course of the disease process, to investigate <u>aqueous humor dynamics</u> in both affected and unaffected eyes, and to attempt to find <u>genetic markers</u> such as <u>HLA and ABO antigens</u> or physical correlates with the disease process.		

Project Description:

Objectives: The objectives of the study are to develop a panel of patients with progressive essential iris atrophy and to study these patients to determine factors which may aid in understanding the pathophysiology of the disease process and to study the natural history of this disease. Measurements of aqueous humor dynamics, assessment of genetic markers such as HLA and ABO antigens and physical correlates, and iris fluorescein angiography to determine the role of the vasculature will be carried out.

Methods Employed: During the course of the evaluation the following procedures are performed:

- Complete family history with detailed pedigree
- Best corrected visual acuity with manifest refraction
- Slit lamp examination
- Visual field examination (Goldmann 1<sub>2e</sub> and I<sub>4e</sub>)
- Photography of iris and iris transillumination
- Gonioscopy and goniphotography
- Iris fluorescein angiography and photography
- Baseline tonography
- A complete medical and dental evaluation
- Dilated ophthalmoscopic examination
- Stereophotographs of the optic nervehead

Major Findings: Histopathologic and electron microscopic study of iris and trabecular meshwork tissue has not indicated any clues to the pathogenesis of the disease process.

An ultrathin corneal contact lens is useful in certain patients to prevent recurrent rupture of corneal bullae.

Significance to Biomedical Research and the Program of the Institute: These data may contribute to an understanding of pathophysiologic factors involved in the rare entity of progressive essential iris atrophy. In addition, a careful study of the progression of the disease from the earliest signs will clarify the significance of corneal involvement and the status of outflow channels which may add to the understanding of the mechanism of glaucoma.

Proposed Course: The project will continue for four more years.

NEI Research Program: Glaucoma--Etiology of Glaucoma (Developmental Glaucoma/Secondary Glaucomas)

Publications:

Kaiser-Kupfer M, Kuwabara T, Kupfer C: Progressive bilateral essential iris atrophy. Am J Ophthalmol 83:340, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00060-02 CB
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less)  Visual Function and Ocular Pigmentation in Albinism		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  PI: Muriel I. Kaiser-Kupfer M.D. Senior Staff Ophthalmologist CB NEI Other: None		
COOPERATING UNITS (if any)  None		
LAB/BRANCH Clinical Branch		
SECTION		
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20014		
TOTAL MANYEARS: .20	PROFESSIONAL: .15	OTHER: .05
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)  Patients with <u>hypomelanotic disorders</u> such as <u>ocular albinism</u> , <u>oculocutaneous albinism</u> , <u>Chediak-Higashi Disease</u> , <u>Hermansky-Pudlak Syndrome</u> and <u>Iris trans-illumination defects</u> are being recruited to determine visual function and to evaluate its course over time. Family members are evaluated to attempt to determine factors which may identify the heterozygous state.		

Project Description:

Objectives: The objectives of the study are to relate the level of visual function to the amount of ocular pigmentation, especially iris and retinal pigmentation; to correlate the amount of nystagmus with visual acuity and iris pigmentation; to determine whether ocular pigmentation, visual acuity, and nystagmus change with age; and to identify the heterozygous state in family members.

Methods Employed: The following examinations are performed:

- Complete family history with detailed pedigree
- Best corrected visual acuity at near and distance with refraction
- Slit lamp examination
- Nystagmus recording using eye movement monitoring EOG
- Psychophysical testing including D-15 and Munsell 100 hue, rod and cone thresholds
- Dilated ophthalmoscopic examination
- Hair bulb incubation
- Photography to document hair color, eye color, skin color, iris transillumination, disc, and macula

Examination of family members includes:

- Best corrected visual acuity
- Slit lamp examination of iris
- Photography of iris transillumination
- Fundus examination when vision not corrected to 20/20

Major Findings: Examination of patients and family members indicates that the finding of transillumination of the iris may be seen in the absence of recognized albinism. The pattern appears to be punctate and may be present in a diffuse manner or limited to the 6 o'clock sector.

Significance to Biomedical Research and the Program of the Institute: These data may allow identification of the carrier state in albinism which would be of importance in genetic counselling. In addition, it may be possible to determine whether the development of the fovea is abnormal in albinism and if this is the cause of the decreased visual acuity in albinism or whether decreased visual acuity is secondary to hypopigmentation and the resultant light-scatter and glare. In addition, it will be possible to ascertain whether visual acuity improves with age and if this is correlated with changes in pigmentation.

Proposed Course: This project will be continued for five more years.

NEI Research Program: Retinal and Choroidal Diseases--Developmental and Hereditary Disorders

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00013-07 CB								
PERIOD COVERED October 1, 1977 to September 30, 1978										
TITLE OF PROJECT (80 characters or less)  Study of Pharmacodynamics of Various Agents Affecting the Intraocular Pressure										
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: Frank J. Macri</td> <td style="width: 15%;">Ph.D.</td> <td style="width: 33%;">Pharmacologist</td> <td style="width: 19%;">CB NEI</td> </tr> <tr> <td>Other: John Helal</td> <td>M.D.</td> <td>Visiting Scientist</td> <td>CB NEI</td> </tr> </table>			PI: Frank J. Macri	Ph.D.	Pharmacologist	CB NEI	Other: John Helal	M.D.	Visiting Scientist	CB NEI
PI: Frank J. Macri	Ph.D.	Pharmacologist	CB NEI							
Other: John Helal	M.D.	Visiting Scientist	CB NEI							
COOPERATING UNITS (if any)  None										
LAB/BRANCH Clinical Branch										
SECTION										
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20014										
TOTAL MANYEARS: 3.0	PROFESSIONAL: 2.0	OTHER: 1.0								
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS										
SUMMARY OF WORK (200 words or less - underline keywords) <p> <u>Isoproterenol</u>, a B-adrenergic agonist, has been known for years to be effective in decreasing aqueous humor (AH) production in man. Most recently, the B-adrenergic antagonist, <u>timolol</u>, has been found to have effects identical to the agonist. Our studies on intact, anesthetized cats and on enucleated arterially perfused eyes indicate that the action of timolol may not be mediated by <u>B-adrenergic blockade</u>. The precise mechanism of its action is still being pursued.         </p> <p>           The actions of the prostaglandins (PG) E<sub>1</sub>, E<sub>2</sub> and F<sub>2α</sub> as well as of arachidonic acid (AA) were studied in the enucleated, arterially perfused eye. Each of the PGs were found to decrease AH production while AA caused the formation rate of AH to become elevated. A vascular mechanism for each of these responses appears very probable.         </p>										

Project Description:

Objectives: To determine the pharmacodynamics of agents able to alter the intraocular pressure (IOP) with a view to finding more effective compounds and possibly to furthering the understanding of mechanisms which maintain the intraocular pressure.

Methods Employed: Studies were performed, in situ, on eyes of anesthetized cats and on enucleated arterially perfused eyes. In the latter, the perfusate is channeled through the ophthalmic artery to nourish the entire eye, or a ligature is placed around the optic nerve at its insertion, so that only the anterior segment of the eye is perfused. Drugs and other test substances are added to individual bottles of perfusate fluid which can then be introduced into the system by stopcock control. Temperature and rate of arterial flow are easily regulated. The rate of aqueous humor formation was estimated by determining the rate of decay of intracamerally injected  $^{125}\text{I}$  tagged serum albumin.

Major Findings: Timolol does not appear to exert its ocular hypotensive action by blockade of B-adrenergic receptors. In the intact cat, it causes first an increase in the formation of AH; after 45 minutes the AH flow rate and IOP start to decline. The latter responses occur at a time well past that of the initiation of B-adrenergic blockade and therefore appear dissociated. Studies are being pursued, using the enucleated eye preparation, to ascertain the mechanism of timolol's effects.

The prostaglandins  $\text{E}_1$ ,  $\text{E}_2$ , and  $\text{F}_{2\alpha}$  were each found to lower the rate of AH formation. The mechanism of the response is a relaxation of efferent ciliary process blood vessels to decrease ultrafiltration. If tonus is exerted on afferent blood vessels of the ciliary process through a neurogenic mechanism, then the PG's become effective in relieving that vasoconstriction as well. Dilations of both blood vessels may well be moderated by the known ability of these PGs to prevent the release of endogenous norepinephrine. Arachidonic acid (AA) produces an increase in AH formation and in IOP. The AH effect can be blocked by prior administration of indomethacin, but the IOP action is unaffected. The increased production of AH by AA is not due to the formed PGs,  $\text{E}_2$  or  $\text{F}_{2\alpha}$ .

Significance to Biomedical Research and the Program of the Institute: Timolol--It appears as a paradox that timolol, a substance which blocks B-adrenergic receptors, produces responses in the eye identical to those induced by drugs which stimulate the same receptors. Data that we are accumulating suggest that though drugs such as timolol may have B-adrenergic propensities, their action on the eye is mediated by other less-well described mechanisms. These findings are leading to a better understanding of control processes affecting AH production.

Prostaglandins--A substantial step forward was made with the observation that the PGs  $\text{E}_1$ ,  $\text{E}_2$ , and  $\text{F}_{2\alpha}$  can diminish the tonus of both the efferent

or afferent blood vessels of the ciliary processes. The amount of tonus exerted on either of these vessels dictates whether AH formation will be increased or diminished. Our experimental findings with PG's have elucidated a rational mechanism to describe the varied IOP effects noted with the PG's; these findings may also be helpful as a tool to approximate the physiologic control of the tonus of the ciliary process blood vessels.

The full significance of the ability of arachadonic acid to increase AH flow awaits further clarification. The action appears due to a PG precursor.

Proposed Course: We plan to continue with the timolol studies. Determination of its mechanism of action may well lead to a better understanding of the mechanisms which regulate AH production and could lead to possibly better selection of drugs for glaucoma treatment. The PG's and arachadonic acid will be studied further to determine if they may play a physiological role in eye pressure regulation. It is hoped that some investigations into the question of neural control of AH production can also be performed.

NEI Research Program: Glaucoma--Hydrodynamics of the Eye

Publications:

Macri FJ: A proposed ultrafiltrative mechanism for the formation of aqueous humor. Bibl Anat 16:76-79, 1977.

Macri FJ, Cevario SJ: The inhibitory actions of dopamine, hydroxy-amphetamine, and phenylephrine on aqueous humor formation. Exp Eye Res 26:85-89, 1978.

Macri FJ, Cevario SJ: Aqueous humor formation: A proposed mechanism of actions. Arch Ophthalmol (in press).

Macri FJ, Cevario SJ: Clonidine: Effects on aqueous humor formation and intraocular pressure. Arch Ophthalmol (in press).



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE <b>NOTICE OF</b> INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00072-01 CB
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less) Biology of Normal and Abnormal Sensory Retina and Pigmented Epithelium		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: David A. Newsome M.D. Other: Gerald Chader Ph.D.  John Hassell Ph.D. Paul O'Brien Ph.D.	Senior Staff Ophthalmologist Head Section on Retinal and Corneal Metabolism Senior Staff Fellow Research Chemist	CB NEI LVR NEI  CB NEI LVR NEI
COOPERATING UNITS (if any) Laboratory of Vision Research, NEI Hazleton Laboratories		
LAB/BRANCH Clinical Branch		
SECTION		
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20014		
TOTAL MANYEARS: 0.75	PROFESSIONAL: 0.75	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)  Studies were conducted to determine <u>control mechanisms</u> regulating the <u>phago-</u> <u>cytic activity of retinal pigmented epithelium</u> . Cells from normal and <u>retinal</u> <u>dysplastic animals</u> were grown in culture and tested using two different ap- proaches. Radioactively labeled <u>photoreceptor outer segments</u> from normal dogs were incubated with cultures of normal Irish setter, retinal dystrophic Irish setter, normal monkey, chick, and human ocular cells in culture. The outer segments were ingested by all the cells to which they were exposed, not just the pigmented epithelium. The cells from dystrophic animals ingested signifi- cantly fewer labeled outer segments. In another series of experiments, the <u>membrane receptors</u> that have been documented to be on the cells of the reti- culoendothelial system were sought on freshly prepared and cultured cells from normal and dystrophic setters. As judged by <u>rosette formation</u> with <u>immunologically labeled sheep erythrocytes</u> , the <u>Fc receptor</u> and possibly the <u>C3 receptor</u> systems are present.		

Project Description:

Objectives: One of the most critical functions of the retinal pigmented epithelium is the controlled phagocytosis of portions of photoreceptor outer segments. The control mechanisms of this function are poorly understood. This project was designed to explore the phagocytic ability of retinal pigmented epithelium from normal and dysplastic retinas in order to understand better the nature of the retinal disease, a possible model for human disease. A second set of objectives concerns the search for cell surface receptors on the retinal pigmented epithelium. This class of receptors may, as has been shown in the reticuloendothelial system, be critical for phagocytosis.

Methods Employed: Retinal pigmented epithelium cells from several species of donors were cultured by standard techniques from suspensions of freshly collected cells or from explants bearing the pigmented epithelial layer. The resultant cultured colonies of pigmented epithelium were tested for phagocytosis by exposing them to suspensions of bioradiolabeled photoreceptor outer segments harvested from normal animals. Phagocytosis was determined by liquid scintillation counting of the cell layers and confirmed by transmission electron microscopy. Surface receptors were sought using immunologically tagged sheep erythrocytes and determining the frequency of rosette formation by the test cells and the sheep cells.

Major Findings: Both normal and dysplastic Irish setter pigmented epithelium ingested the radiolabeled outer segments. However, the uptake by cells from the abnormal animals was only about half normal as judged by radioactivity in the pigmented epithelial layer. Retinal pigmented epithelium from several species as well as other ocular cells in culture such as corneal and uveal cells also exhibited phagocytosis. Retinal pigmented epithelium cells demonstrated the presence of C3 and possibly Fc receptors by rosette formation with variously tagged sheep erythrocytes in controlled experiments.

Significance to Biomedical Research and the Program of the Institute: Knowledge of the mechanisms and control of phagocytosis of shed photoreceptor outer segments by the retinal pigmented epithelium is crucial to the understanding of normal and diseased retinal function. Data from this project can add to the understanding of the newly available Irish setter model of rod and cone dysplasia, which may augment our knowledge of human diseases and thus help to devise rational attacks on these diseases. The cell surface receptors that participate in immunologic type cell-cell interactions and recognition may be altered in abnormal retinal function. Knowledge of the normal and abnormal immunologic status of the retinal pigmented epithelium will aid in devising therapies based on manipulation of this system.

Proposed Course: Cultures of pigmented epithelium from various species and collections of fresh cells will continue to be made so that the cell surface of normal and abnormal pigmented epithelia can be studied further.

NEI Research Program: Retinal and Choroidal Diseases--Visual Cells and Pigment Epithelium

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00075-01 CB																														
PERIOD COVERED October 1, 1977 to September 30, 1978																																
TITLE OF PROJECT (80 characters or less) Immune Functions in Ocular Diseases of Obscure Etiology																																
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																																
<table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 30%;">Robert Nussenblatt</td> <td style="width: 15%;">M.D.</td> <td style="width: 25%;">Clinical Associate</td> <td style="width: 10%;">CB</td> <td style="width: 10%;">NEI</td> </tr> <tr> <td>Other:</td> <td>Elmer J. Ballintine</td> <td>M.D.</td> <td>Clinical Director</td> <td>CB</td> <td>NEI</td> </tr> <tr> <td></td> <td>David BenEzra</td> <td>M.D., Ph.D.</td> <td>Visiting Scientist</td> <td>CB</td> <td>NEI</td> </tr> <tr> <td></td> <td>Jane Blackman</td> <td>M.D.</td> <td>Senior Staff Fellow</td> <td>CB</td> <td>NEI</td> </tr> <tr> <td></td> <td>Igal Gery</td> <td>Ph.D.</td> <td>Visiting Scientist</td> <td>LVR</td> <td>NEI</td> </tr> </table>			PI:	Robert Nussenblatt	M.D.	Clinical Associate	CB	NEI	Other:	Elmer J. Ballintine	M.D.	Clinical Director	CB	NEI		David BenEzra	M.D., Ph.D.	Visiting Scientist	CB	NEI		Jane Blackman	M.D.	Senior Staff Fellow	CB	NEI		Igal Gery	Ph.D.	Visiting Scientist	LVR	NEI
PI:	Robert Nussenblatt	M.D.	Clinical Associate	CB	NEI																											
Other:	Elmer J. Ballintine	M.D.	Clinical Director	CB	NEI																											
	David BenEzra	M.D., Ph.D.	Visiting Scientist	CB	NEI																											
	Jane Blackman	M.D.	Senior Staff Fellow	CB	NEI																											
	Igal Gery	Ph.D.	Visiting Scientist	LVR	NEI																											
COOPERATING UNITS (if any)  Laboratory of Vision Research, NEI																																
LAB/BRANCH Clinical Branch																																
SECTION																																
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20014																																
TOTAL MANYEARS: 2.0	PROFESSIONAL: 2.0	OTHER: 0.0																														
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																																
SUMMARY OF WORK (200 words or less - underline keywords) <u>In vitro macrophage and lymphocyte functions in toxoplasmosis, presumed ocular histoplasmosis, pars planitis, Behcet's Disease, ocular sarcoid, and Wegener's granulomatosis</u> are being studied. The studies are carried out in a simple masked method. Correlations between test results and the clinical condition are being sought and possibly utilized as a guide for specific immunologic therapy. In addition, the test results could shed light on the basic mechanisms of the ocular inflammatory response.																																

Project Description:

Objectives: It is our objective to investigate several immunologic factors that may relate to the predisposition, cause, and ocular chronicity of these diseases. An attempt is being made to see if these patients are sensitized to ocular antigens. We wish to investigate whether uveitis and the other ocular changes seen in these diseases are a manifestation of an alteration in the immune system with regard to lymphocyte and macrophage reactivity and how these alterations relate to the clinical features of these ocular entities. It is our hope that with a clearer idea of basic mechanisms, a more rational approach to therapy can be developed.

Methods Employed: The ophthalmic examination of all patients includes slit lamp examination, visual field, and fluorescein angiography. Lymphocyte cultures are prepared using the microculture technique, where they are tested against various ocular extracts, rod outer segment preparations, S-antigen, and various mitogens in order to assess their general reactive state. The capillary migration system is used to evaluate migration inhibition. Both tests are considered the in vitro equivalent of the anamnestic response in vivo. Suppressor cell activity is evaluated by the use of sub-optimal doses of the mitogen Concanavalin A in culture, as reported by Bresnihan and Jasin (J Clin Invest 59:109, 1977). Macrophage activity is studied by examining their phagocyte capabilities and production of lymphocyte activating factor.

Major Findings: This work is in a preliminary phase, and no conclusive results have been obtained.

Significance to Biomedical Research and the Program of the Institute: Uveitis is the cause of approximately 5% of legal blindness and 4.5% of severe visual impairment in the United States. In addition, the incidence of ocular involvement in sarcoid is from 17 to 64%, and in Wegener's granulomatosis 41 to 43%. The exact role that the cellular immune response plays in these ophthalmic and systemic conditions remains to be defined better. A clearer idea of the basic mechanisms involved may lead to the prediction of whom with sarcoid and Wegener's granulomatosis will develop ocular involvement, who will develop a chronic form of the ocular disease, and a rational therapeutic hypothesis, such as a trial of immunomodulating agents. Elucidation and treatment of inflammatory conditions of the eye are interests of the National Eye Institute.

Proposed Course: This study will continue with the possible addition of further immunologic evaluations, such as HLA typing, in order to evaluate these patients better.

NEI Research Program: Retinal and Choroidal Diseases--Inflammatory Disorders

Publications:

BenEzra D, Nussenblatt R: Ocular manifestations of Behcet's disease. J Oral Pathol (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE  
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF  
HEALTH, EDUCATION, AND WELFARE  
PUBLIC HEALTH SERVICE  
NOTICE OF  
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00073-01 CB

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Tissue Specificity of Ocular Antigens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER  
PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert Nussenblatt	M.D.	Clinical Associate	CB	NEI
Other:	Igal Gery	Ph.D.	Visiting Scientist	LVR	NEI
	David BenEzra	M.D., Ph.D.	Visiting Scientist	CB	NEI

COOPERATING UNITS (if any)

Laboratory of Vision Laboratory, NEI

LAB/BRANCH

Clinical Branch

SECTION

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The cellular immune response against extracts from the individual corneal layers and lens was studied in guinea pigs and rabbits. Purified T-cells from guinea pig peritoneal exudates and rabbit popliteal lymph nodes were used. Employing the blast transformation phenomenon, which is considered the in vitro counterpart to the anamestic response in vivo, a comparison was made as to the relative immunogenicity and antigenicity of various ocular extracts. Epithelium was found to be a strong immunogen in vivo and antigen in vitro, while endothelium was least active using the same criteria. Stroma showed intermediate activities. Species specific antigens were found in all corneal layers. Strong organ specificity was exhibited by the lens.

Project Description:

Objectives: The cellular immune response is now thought to play a major role in chronic ophthalmic disorders. This project was designed to evaluate this response in relation to extracts from various anterior ocular structures. In doing so, the reactivity and cross-reactivity of these extracts were assessed.

Methods Employed: Human, monkey, guinea pig, and rabbit corneas and lenses are dissected. Extracts of each are prepared for both immunization and use in an in vitro lymphocyte culture system. From immunized guinea pigs, pure T-cell cultures are obtained by passing peritoneal exudate cells over a rayon wool column, with the nonadherent cells being almost purely T. Popliteal lymph nodes were taken from immunized rabbits. After culturing lymphocytes from immunized animals for three to five days in the presence of specific antigens, tritiated thymidine is added before harvesting the cells. Measuring the amount of DNA precursor uptake is a way to assess the response of the cells to the antigens tested.

Major Findings: Of the corneal layers, the epithelium was found to be the best immunogen in vivo and antigen in vitro, while the endothelial-Descemet's layers showed the lowest capacities with both criteria. The stroma was intermediately active. All three layers contained species-specific antigens, which cross-reacted with rabbit kidney. A good cross-reactivity was found between epithelial and lens extracts, but very little or no shared antigenicity was noted between the lens and stroma. In addition, no cross-reactivity was seen between purified lens crystallins and corneal epithelium. Rabbit corneal epithelium and stroma also cross-reacted with monkey or human corneal extracts, suggesting the existence of corneal-specific antigen(s). An apparent organ specificity was exhibited by lens extracts.

Significance to Biomedical Research and the Program of the Institute: The immunologic characteristics of ocular tissue have been the subject of much investigation. Most studies, however, have dealt with the humoral response to ocular antigens. It is now clear that the cellular immune response plays the major role in causing experimental uveitis and corneal graft rejection. A clearer picture of this response, in relation to the eye, will add to an understanding of basic mechanisms and possibly lead to a more rational form of therapy.

Proposed Course: We have begun to study immunologically active components of ocular tissues in animal models. At present, the cellular immune response to the lens crystallins and retinal rod outer segments is being studied. This work will be done in conjunction with our clinical studies dealing with ocular inflammatory disease.

NEI Research Program: Corneal Diseases--Corneal Transplantation and Stromal Injury and Repair

Publications:

Nussenblatt R, Gery I, BenEzra D: The specificity of ocular antigens, in The Second International Symposium in Ocular Immunology and Immunopathology. Paris, Masson and Co (in press).



SMITHSONIAN SCIENCE INFORMATION EXCHANGE  
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF  
HEALTH, EDUCATION, AND WELFARE  
PUBLIC HEALTH SERVICE  
NOTICE OF  
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00050-02 CB

PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Aqueous Humor Flow Measurement by Fluorophotometry

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER  
PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Jonathan E. Pederson	M.D. Clinical Associate	CB	NEI
Other:	Douglas E. Gaasterland	M.D. Senior Staff Ophthalmologist	CB	NEI
	Lessie McCain	R.N. Clinical Technician	CB	NEI
	Helen MacLellan	M.S. Biologist	CB	NEI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Clinical Branch

SECTION

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

0.9

PROFESSIONAL:

0.3

OTHER:

0.6

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The aqueous humor flow in humans has been measured by determining the rate of loss of fluorescein from the eye after iontophoresis into the cornea. In one group of patients, this has been compared to the aqueous humor flow calculated from tonographic results. Reproducibility and symmetry of measurements have been tested in normal volunteers and in patients with glaucoma. There are several sources of errors in fluorophotometry; these have been studied.

Project Description:

Objectives: This project is designed to measure directly aqueous humor flow in humans. This will be compared to calculated aqueous humor flow. The symmetry and reproducibility of measurements of aqueous humor flow in the two eyes of normal volunteers and of patients with either ocular hypertension or glaucoma are to be studied.

Methods Employed: A cylindrical piece of polyacrilamide gel is saturated with fluorescein solution. The gel is touched to the cornea, and fluorescein is deposited due to a small current provided by a dry cell battery. A photomultiplier tube with appropriate filters, mounted on a slit lamp biomicroscope, measures the total amount of fluorescein in the eye as well as the aqueous concentration. Illumination is provided by a chopped light source. The photomultiplier tube signal is fed to a tuned amplifier. The rate of loss of fluorescein from the eyes as a function of time yields the flow rate of aqueous humor.

Major Findings:

I. Young normal volunteers

In young normal volunteers, the measured rate of aqueous flow as indicated by the turnover of fluorescein solution is three to five times more than the calculated rate of aqueous flow. The turnover of fluorescein is affected by the site on the cornea of fluorescein iontophoresis. Turnover is faster when iontophoresis is placed next to the limbus.

II. Patients with open-angle glaucoma

The rate of aqueous flow indicated by turnover of fluorescein is less in untreated glaucoma patients, with pressures between 35 and 45 mmHg, than in normal volunteers of the same age. However, in the glaucoma patients, aqueous flow indicated by fluorescein is twice the flow indicated by calculation from tonographic results.

Significance to Biomedical Research and the Program of the Institute:

The aqueous humor flow rate is a primary determinant of the intraocular pressure. An accurate, safe, reproducible, noninvasive, direct determination of the flow in humans under normal and pathological conditions will lead to increased understanding of glaucoma and hypotony.

Proposed Course: The studies will continue.

NEI Research Program: Glaucoma--Optic Nerve and Vision Changes in Glaucoma

Publications:

Pederson JE, Gaasterland DE, MacLellan H: Accuracy of aqueous humor flow determination by fluorophotometry. Invest Ophthalmol Visual Sci 17:190, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00078-01 CB																					
PERIOD COVERED October 1, 1977 to September 30, 1978																							
TITLE OF PROJECT (80 characters or less)  Biochemistry and Morphology of Human Corneal Dystrophies and Degenerations																							
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">Merlyn Rodrigues</td> <td style="width: 15%;">M.D.</td> <td style="width: 30%;">Medical Officer</td> <td style="width: 5%;"></td> <td style="width: 5%;">CB</td> <td style="width: 10%;">NEI</td> </tr> <tr> <td>Other:</td> <td>David A. Newsome</td> <td>M.D.</td> <td>Senior Staff Ophthalmologist</td> <td></td> <td>CB</td> <td>NEI</td> </tr> <tr> <td></td> <td>John R. Hassell</td> <td>Ph.D.</td> <td>Staff Fellow</td> <td></td> <td>CB</td> <td>NEI</td> </tr> </table>			PI:	Merlyn Rodrigues	M.D.	Medical Officer		CB	NEI	Other:	David A. Newsome	M.D.	Senior Staff Ophthalmologist		CB	NEI		John R. Hassell	Ph.D.	Staff Fellow		CB	NEI
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Other:	David A. Newsome	M.D.	Senior Staff Ophthalmologist		CB	NEI																	
	John R. Hassell	Ph.D.	Staff Fellow		CB	NEI																	
COOPERATING UNITS (if any)  Department of Ophthalmology, University of Iowa Dermatology Branch, NCI																							
LAB/BRANCH Clinical Branch																							
SECTION Section on Clinical Pathology																							
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20014																							
TOTAL MANYEARS: 1.5	PROFESSIONAL: 1.0	OTHER: 0.5																					
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SUMMARY OF WORK (200 words or less - underline keywords)  <p> <u>Human corneal dystrophies</u> and selected <u>corneal degenerations</u> which have been documented carefully in the patient are then, as specimens following <u>corneal transplantations</u>, studied in the laboratory in an effort to elucidate patho-genetic mechanisms. Morphologic examination by light and electron microscopy has provided further insight into <u>cell-cell relationships</u> in the normal and diseased states and about <u>abnormal extracellular deposits</u>, such as the newly described crystals in a patient with benign monoclonal gammopathy. Tissue and cell culture studies have revealed the in vitro proliferative patterns of corneal cells, including the <u>epithelialization of the endothelial layer</u> in corneas of patients with <u>posterior polymorphous dystrophy</u>. The presence and production of <u>collagen</u> and <u>glycoconjugates</u> and of <u>collagenase</u> has been probed with immunofluorescent, electrophoretic, and chromatographic procedures, with emphasis to date on abnormalities of collagen production and collagenase activity in <u>keratoconus</u>.         </p>																							

Project Description:

Objectives: Visually disabling human corneal diseases have been studied in the past primarily by clinical means, occasionally coupled with morphological analysis. Pathogenetic mechanisms remain obscure. This study will attempt to combine detailed clinical and genetic observations in order to understand and classify the processes of corneal opacification that result in impaired vision.

Methods Employed: Corneal specimens from transplant patients were divided into portions and used separately for light, scanning electron, and transmission electron microscopy. These data provided insight into the morphological appearance of the cells and the extracellular materials of the corneal layers. Other portions of the surgical specimens were placed into tissue and cell culture to allow examination of the morphology and biosynthetic activities of the cells of the three corneal layers. Indirect immunofluorescence has shown the range of collagen types present in normal and abnormal tissue. Column chromatography and electrophoresis have provided information about the collagen and glycoconjugate biosynthetic patterns of both normal and abnormal tissues.

Major Findings: Several cases of hereditary posterior polymorphous corneal dystrophy had an admixture of epithelial and endothelial cells on Descemet's membrane which is normally lined by a monolayer of endothelial cells. Descemet's membrane also showed irregular thinning, a probable cause of the clinically seen opacities. Corneal tissue with lattice dystrophy possessed autofluorescence with ultraviolet light. Cells cultured from ocular tissues of a patient with lattice dystrophy have tested positive for amyloid and will be evaluated extensively to determine the nature of this abnormal product. Keratoconus specimens demonstrated the same range of collagen types as normal cornea, with the exception of a thinned and occasionally missing Descemet's membrane and Bowman's layer. Radioactive labeling experiments on cultured cells from these corneas have demonstrated an elevated production of Type III collagen and of collagenase as compared with normal. In case of posterior keratoconus, an abnormal Descemet's membrane and endothelium was shown by transmission electron microscopy. The presence of stromal crystals bilaterally in two patients was correlated with the presence of kappa light chains of immunoglobulin in the serum of one and in the urine of the other. The corneal findings were crucial to the diagnosis of the systemic disease.

Significance to Biomedical Research and the Program of the Institute: The mechanisms of opacification and destruction of the cornea in a variety of human diseases must be understood for the improved diagnosis and classification of these entities. This would also permit a rational basis for the appropriate treatment of these visually disabling processes. A thorough knowledge of the genetic component of these disorders, if any, will aid in more effective and complete genetic counseling.

Proposed Course: Patient material will be entered into this combined study as it becomes available. Emphasis will be placed on elucidating the

hereditary posterior polymorphous dystrophy, keratoconus, and lattice and granular dystrophies. The use of immunological techniques will be expanded to a wider variety of specimens.

NEI Research Program: Corneal Diseases--Corneal Edema, Dystrophies, and Inherited Disorders

Publications:

Rodrigues M, Krachmer J, Miller S, Newsome D: Bilateral corneal crystalline deposits in benign monoclonal gamopathy. Arch Ophthalmol (in press).

Krachmer J, Rodrigues M: Posterior keratoconus. Arch Ophthalmol (in press).

Waring G, Rodrigues M, Laibson PR: Corneal dystrophies. A clinico-pathologic study. Surv Ophthalmol (in press).

Rodrigues M, Waring G: Anterior and posterior corneal dystrophies, in Klintworth G, Garner A (eds): Pathobiology of Ocular Diseases. New York, Marcel Dekker Co (in press).



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00056-02 CB																		
PERIOD COVERED October 1, 1977 to September 30, 1978																				
TITLE OF PROJECT (80 characters or less)  Transport Mechanisms in the Ciliary Epithelium																				
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																				
<table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 35%;">Richard A. Stone</td> <td style="width: 10%;">M.D.</td> <td style="width: 30%;">Clinical Associate</td> <td style="width: 10%;">CB</td> <td style="width: 5%;">NEI</td> </tr> <tr> <td>Other:</td> <td>Elmer J. Ballintine</td> <td>M.D.</td> <td>Clinical Director</td> <td>CB</td> <td>NEI</td> </tr> <tr> <td></td> <td>Richard Weiblinger</td> <td>B.S.</td> <td>Biologist</td> <td>CB</td> <td>NEI</td> </tr> </table>			PI:	Richard A. Stone	M.D.	Clinical Associate	CB	NEI	Other:	Elmer J. Ballintine	M.D.	Clinical Director	CB	NEI		Richard Weiblinger	B.S.	Biologist	CB	NEI
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Other:	Elmer J. Ballintine	M.D.	Clinical Director	CB	NEI															
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COOPERATING UNITS (if any)  None																				
LAB/BRANCH Clinical Branch																				
SECTION																				
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20014																				
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SUMMARY OF WORK (200 words or less - underline keywords)  Transport of <u>para-aminohippurate</u> and cholic acid by isolated surviving preparations of epithelium from <u>iris</u> and <u>ciliary body</u> of rhesus monkey eyes was demonstrated, and the chemical kinetics of the processes were measured. The kinetics of the uptake of these two substances were affected differently by a series of inhibitors. The results were consistent with the hypothesis that the processes responsible for the accumulation were different for the two substances but shared some common features. The knowledge of how various organic acids are transported in the eye is useful in devising effective schedules for administration of drugs to the eye.																				

Project Description:

Objectives: To determine the mechanisms by which organic acids are abstracted from the aqueous humor and accumulated in iris and ciliary process.

Methods Employed: Rhesus monkey eyes were obtained from animals used by the Bureau of Biologics. Specimens of iris and of ciliary processes were dissected from these eyes and incubated in Tyrode solution. Radiolabelled para-aminohippurate and cholic acid were added to the medium and the rate of accumulation was measured by determining the radioactivity of the tissues after incubation. The effects of inhibitors such as anoxia, cyanide, ouabain, dinitrophenol, probenecid, idopyracet, and hippuric acid on the accumulation were also measured. Equations were derived to express the amount of accumulation with time and used to calculate the rate constants for the accumulation process.

Major Findings: The rate at which the two organic acids accumulate in the ciliary process is about twice the rate at which they accumulate in the iris. This is the first demonstration of the uptake process in the iris as distinct from the ciliary processes and the first systematic investigation in tissues from monkey eye. There are two parallel transport processes: one mainly transports cholic acid, the other, mainly para-aminohippurate; but the processes overlap in their specificity.

Significance to Biomedical Research and the Program of the Institute: Elucidation of the transport of substances into and out of the aqueous humor is fundamental to an understanding of drug transport mechanisms in the eye and can provide a basis for investigating and understanding glaucoma and its response to treatment with drugs.

Proposed Course: The study has been completed.

NEI Research Program: Glaucoma--Hydrodynamics of the Eye

Publications: None

Laboratory of Vision Research



ANNUAL REPORT  
NATIONAL EYE INSTITUTE  
October 1, 1977 - September 30, 1978

REPORT OF THE CHIEF, LABORATORY OF VISION RESEARCH  
Jin H. Kinoshita, Ph.D.

In the Laboratory of Vision Research, there is considerable diversification of research interests; however, one research subject that is receiving considerable attention from a number of LVR investigators is vitamin A. The importance of this vitamin in ocular tissues is obvious because its derivative plays a key role in vision as the light and color capturing component of visual pigments. In addition, vitamin A or its derivative is essential for a healthy cornea. Vitamin A deficiency results in night blindness and if severe enough leads to xerophthalmia and keratomalacia involving the cornea. LVR investigators are examining the various basic aspects of vitamin A metabolism to see if any leads can be uncovered to give insight to clinical problems associated with vitamin A abnormalities. One of the interesting findings of these studies is the relationship between vitamin A and vitamin E. It appears that vitamin E functions by protecting against oxidation of vitamin A and thereby retarding the loss of vitamin A from the pigment epithelium. Vitamin E deficiency leads to the accumulation of the aging pigment, lipofuscin, in several tissues, to the degeneration of the outer segments, and loss of photoreceptor cells. These results indicate that vitamin E, along with vitamin A, plays a vital role in maintaining the normal state of ocular tissues.

The presence of specific receptors or binding proteins for vitamin A has been demonstrated in the retina, pigment epithelium, and cornea. In a patient with advanced retinitis pigmentosa, one of the receptors for vitamin A was missing in the retina. Receptors for vitamin A (retinol) and for retinoic acid were found in cultured human retinoblastoma cells. The distribution of these receptors in the tumor cells indicated that the retinol receptor was in the cytoplasm while the retinoic acid receptor was primarily found in the nucleus. Further work is needed to define precisely the roles of these receptors in health and disease of ocular tissues.

Another interesting feature that emerges in reviewing the annual reports of the research projects is the obvious extensive collaboration that exists between the LVR investigators and scientists from other intramural programs and eye research laboratories throughout the country. The intramural program at NIH is richly endowed with talented scientists in many disciplines. Thus the collaboration with other intramural scientists is taking advantage of expertise available at NIH which frequently adds new dimensions in attacking a research problem. For example, the vitamin A deficiency studies would have been more difficult if not impossible to conduct without the collaboration of the expert nutritionist, Dr. Bieri of NIAMDD. The collaboration with scientists outside NIH has also been fruitful and has resulted in accelerating the progress of a number of LVR research projects. In addition, the interactions keep the NIH scientists from becoming isolated and make them aware of their unique status as part of the vision research community. A salutary effect of these collaborations is that it brings the extramural community and the NEI closer together.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00003-06 LVR																									
PERIOD COVERED October 1, 1977 to September 30, 1978																											
TITLE OF PROJECT (80 characters or less)  Cataracts																											
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																											
<table style="width: 100%; border: none;"> <tr> <td style="width: 35%;">PI: Jin H. Kinoshita</td> <td style="width: 15%;">Ph.D.</td> <td style="width: 30%;">Chief</td> <td style="width: 10%;">LVR</td> <td style="width: 10%;">NEI</td> </tr> <tr> <td>Other: Henry N. Fukui</td> <td>Ph.D.</td> <td>Senior Staff Fellow</td> <td>LVR</td> <td>NEI</td> </tr> <tr> <td>Suguru Fukushi</td> <td>M.D.</td> <td>Visiting Scientist</td> <td>LVR</td> <td>NEI</td> </tr> <tr> <td>Peter Kador</td> <td>Ph.D.</td> <td>Staff Fellow</td> <td>LVR</td> <td>NEI</td> </tr> <tr> <td>Lorenzo Merola</td> <td></td> <td>Chemist</td> <td>LVR</td> <td>NEI</td> </tr> </table>			PI: Jin H. Kinoshita	Ph.D.	Chief	LVR	NEI	Other: Henry N. Fukui	Ph.D.	Senior Staff Fellow	LVR	NEI	Suguru Fukushi	M.D.	Visiting Scientist	LVR	NEI	Peter Kador	Ph.D.	Staff Fellow	LVR	NEI	Lorenzo Merola		Chemist	LVR	NEI
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COOPERATING UNITS (if any)  None																											
LAB/BRANCH Laboratory of Vision Research																											
SECTION Section on Biochemistry																											
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20014																											
TOTAL MANYEARS: 3.5	PROFESSIONAL: 2.5	OTHER: 1.0																									
CHECK APPROPRIATE BOX(ES)																											
<input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER																											
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																											
SUMMARY OF WORK (200 words or less - underline keywords)																											
<p>Two types of cataracts are currently being investigated. The <u>sugar cataracts</u> are initiated by the action of the enzyme, <u>aldose reductase</u>. Effective means of delaying the onset of this type of cataract are being developed.</p> <p>The second type of cataract studied is the <u>hereditary Nakano mouse cataract</u>. This cataract appears to be caused by a defective cation pump mechanism.</p>																											

Project Description:

Objectives: To study the mechanism of formation of cataracts in experimental animals and to explore possible means by which these cataracts can be prevented.

Methods Employed: Sugar cataracts can be induced in experimental animals by making them diabetic with appropriate chemical agents, or by making them galactosemic or xylosemic with a diet enriched with galactose or xylose. Another approach to studying cataracts is to employ animal models. We have developed a colony of a Nakano mouse strain with hereditary cataracts.

Major Findings: Studies of two types of cataracts are being undertaken. In sugar cataracts, galactosemic and diabetic, it has been established that the enzyme aldose reductase triggers the cataractous process. Treatment of animals which are galactosemic or diabetic with an aldose reductase inhibitor effectively delays the onset of cataracts.

The second form of cataract is a hereditary type observed in the Nakano mouse strain. This is an osmotic cataract caused by a defect in cation pump mechanism. The apparent deficiency in the Na-K ATPase appears to be due to an endogenous inhibitor of the enzyme.

Since the enzyme aldose reductase appears to be involved in the process that initiates sugar cataract formation, efforts are being directed toward the discovery of more potent inhibitors of aldose reductase in hope of developing methods of preventing this type of cataract. Studies have shown that flavonoids are potent inhibitors of aldose reductase, the enzyme which appears to initiate the formation of diabetic and galactosemic cataracts. These studies have been extended further to explore the feasibility of using these compounds for the prevention or delay of cataracts in intact diabetic animals. In the past, the difficulty associated with such experiments is the long time taken by most diabetic animals to develop frank lenticular opacity. We found that from this point of view, the Octodon degu, a South American rodent, is a convenient experimental model. It was observed that aldose reductase activity in the degu lens is much higher than that of any other animal lenses. The degu lens develops frank opacity as early as one week following the induction of diabetes by streptozotocin. Administration of flavonoids to diabetic degus leads to a substantial reduction in the level of lens sorbitol as compared to controls. We have now evidence that quercitrin fed orally is effective in actually delaying the appearance of opacity in diabetic degus, thus lending further support to the polyol theory of sugar cataract formation. Recently it was shown that quercitrin was mutagenic in a bacterial test system so the potential clinical usefulness of quercitrin is questionable.

In evaluating the potency of inhibitors, aldose reductase (A.R.) from animal lenses has been used. Recently, we found that the relative potency of inhibitors against rat lens A.R. may not correlate with their potency against human placental A.R. For example, quercitrin, an active inhibitor against rat lens A.R. was less active against human placental A.R. Conversely, several

chromone-2-carboxylic acids which were less active against rat lens A.R. were substantially more active against human A.R. Limited experiments with human lens A.R. have been conducted. From the results, it appears that the amount of inhibition against human lens A.R. is more consistent with that observed with human placental enzyme rather than with rat lens enzyme. These findings indicated that although it is more convenient to screen compounds for A.R. inhibitor activity with animal enzyme, evaluation for potential clinical use requires the use of human aldose reductase.

Another tissue in which aldose reductase is implicated in diabetes may be in the cornea. A delay in the re-epithelialization of a denuded corneal stroma in diabetics is a recognized clinical manifestation. In studying this problem we wondered about the possibility of the involvement of aldose reductase in the diabetic corneal epithelium.

Enzyme assays have revealed that aldose reductase is present in the corneal epithelium. It was inhibited by various compounds that inhibited lens aldose reductase. Incubation of sheets of corneal epithelium from bovine eyes in high concentrations of sugar led to accumulation of sugar alcohol similar to that observed in the lens.

The epithelium of the cornea was removed from the eyes of diabetic and normal rats. The rate of regeneration of the epithelial layer was followed. The re-epithelialization of the corneas of diabetic rats required a longer period than that required for corneas of normal rats.

The effect of aldose reductase inhibitors in the restoration of the epithelial layer of the denuded cornea was also examined. The corneal epithelium was removed from both eyes of a diabetic rat. One eye was topically treated with a solution containing Alrestatin, an aldose reductase inhibitor. The control eye was given the same solution without the inhibitor. The results revealed that in the eye treated with Alrestatin the epithelium grows over the denuded corneal stroma much more quickly than it does in the untreated control eye. A second aldose reductase inhibitor, structurally different from Alrestatin, also had similar beneficial effects.

Another cataract extensively studied is a hereditary type that occurs in a certain mouse strain. In Nakano mice a pinhead opacity in the lens nucleus appears 3 weeks after birth. Just prior to the appearance of the cataract there is a sudden increase in lens hydration that is related to an elevation in sodium ion content. The inability of the lens to extrude  $\text{Na}^+$  apparently is due to a defect in the cation pump. Probably related to this is a decrease in the Na-K ATPase activity. The explanation for the depressed ATPase activity appears to be due to the presence of an inhibitor of the enzyme in the Nakano lens.

The inhibitor of Na-K ATPase can be demonstrated in extracts of the Nakano lens but not in normal mouse lens. Fractionation by gel filtration reveals that the inhibitor is found in fractions not associated with the major lens crystallins but with low molecular weight substances. The method used to quantitate the level of inhibitor is to deproteinize the lens with sulfosalicylic acid followed

by dialysis. The inhibitor appears to affect only the Na-K ATPase and not the nonspecific Mg-ATPase. The inhibitor is effective against Na-K ATPase prepared from mouse, rat, and calf lenses as well as calf retina and brain.

The inhibitor is inactivated by carboxypeptidase and leucine amino peptidase. The polypeptide has a molecular weight around 8,000 and a basic charge at neutral pH. It is remarkably stable to low and high pHs and can withstand 100°C without loss of activity. A snake venom cytotoxic factor has many properties similar to the Nakano mouse cataract factor. Both the snake venom cytotoxic factor and Nakano cataract factor are positively charged polypeptides of the 6-10,000 M.W. class, are resistant to acid and base, stable in boiling water and are inhibitors of Na-K ATPase.

Significance to Biomedical Research and the Program of the Institute:

Cataract is one of the major causes of blindness throughout the world. Even though vision can be corrected by appropriate surgery, loss of vision because of cataracts presents a problem. It is hoped that this type of study on sugar cataracts may serve as a model by which other mechanisms of cataract development can be uncovered, and also provide means of preventing cataracts. The terminal stages of these sugar cataracts may have features common to other forms of cataracts. Even though the initial phase of cataract development may be different in the other forms of cataract, it appears that the terminal stages are quite similar.

Proposed Course: This project will be continued.

NEI Research Program: Cataract--Diabetic Cataract/Congenital, Metabolic, and Genetic Cataracts

Publications:

Fukui HN, Iwata S, Epstein DL, Merola LO: Cataractogenic effects of a boron hydride disulfide compound. Invest Ophthalmol Visual Sci 16:654-657, 1977.

Fukui HN, Merola LO, Kinoshita JH: A possible cataractogenic factor in the Nakano mouse lens. Exp Eye Res 26:477-485, 1978.

Tsunematsu Y, Fukui HN, Kinoshita JH: Studies on primary cultures of adult lens cells from normal and cataractous mice. Exp Eye Res (in press).

Piatigorsky J, Fukui HN, Kinoshita JH: Differential synthesis, degradation and leakage of protein in an inherited cataract and in the normal lens cultures with ouabain. Nature (in press).

Kador PF, Sharplins NE: Structure-activity studies of aldose reductase inhibitors containing the 4-oxo-chromen ring system. Biophys Chem 8:81-85, 1978.

Kador PF, Merola LO, Kinoshita JH: Differences in the susceptibility of various aldose reductases to inhibition. Doc Ophthalmol (in press).

Kador PF, Kinoshita JH: Phospholipid effects on the rat lens transport systems. Exp Eye Res (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00136-06 LVR																
PERIOD COVERED October 1, 1977 to September 30, 1978																		
TITLE OF PROJECT (80 characters or less)  Chemistry and Metabolism of the Lens																		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																		
<table style="width: 100%; border: none;"> <tr> <td style="width: 30%;">PI: Jin H. Kinoshita</td> <td style="width: 10%;">Ph.D.</td> <td style="width: 30%;">Chief</td> <td style="width: 30%; text-align: right;">LVR NEI</td> </tr> <tr> <td>Other: Paul Russell</td> <td>Ph.D.</td> <td>Staff Fellow</td> <td style="text-align: right;">LVR NEI</td> </tr> <tr> <td>Samuel Zigler</td> <td>Ph.D.</td> <td>Staff Fellow</td> <td style="text-align: right;">LVR NEI</td> </tr> <tr> <td>Deborah Carper</td> <td></td> <td>Biologist</td> <td style="text-align: right;">LVR NEI</td> </tr> </table>			PI: Jin H. Kinoshita	Ph.D.	Chief	LVR NEI	Other: Paul Russell	Ph.D.	Staff Fellow	LVR NEI	Samuel Zigler	Ph.D.	Staff Fellow	LVR NEI	Deborah Carper		Biologist	LVR NEI
PI: Jin H. Kinoshita	Ph.D.	Chief	LVR NEI															
Other: Paul Russell	Ph.D.	Staff Fellow	LVR NEI															
Samuel Zigler	Ph.D.	Staff Fellow	LVR NEI															
Deborah Carper		Biologist	LVR NEI															
COOPERATING UNITS (if any)  None																		
LAB/BRANCH Laboratory of Vision Research																		
SECTION Section on Biochemistry																		
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20014																		
TOTAL MANYEARS: 3.5	PROFESSIONAL: 3.5	OTHER: 0.0																
CHECK APPROPRIATE BOX(ES)																		
<input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER																		
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																		
SUMMARY OF WORK (200 words or less - underline keywords)																		
<p>           To study the specific properties of lens cells with particular reference to <u>congenital cataracts</u>, <u>tissue culture</u> methods are being developed. The cell culture of <u>lens epithelium</u> has progressed so that <u>cloned cell lines</u> from mouse lens were obtained. Certain differentiated characteristics of lens cells have been maintained by the cultured epithelial cells. <u>Gamma crystallin</u>, normally associated with the differentiated fiber cells, has been demonstrated using <u>slab gel electrophoresis</u> and <u>immunodiffusion</u>. In addition, proteins synthesized by lens epithelial cells in culture have been studied using <u>autoradiographic methods</u>. <sup>125</sup>I <u>radioimmunoassays</u> for alpha, beta, and <u>gamma crystallin</u> have been developed to check for these lens-specific proteins. Studies of cells cultured from normal mouse lens and mouse lens from a strain with <u>hereditary cataracts</u> have been performed with <u>scanning and transmission electron microscopy</u> and <u>histochemistry</u>.         </p>																		

Project Description:

Objectives: To study lens epithelial cells in the defined environment of tissue culture in order to gain insight into metabolic changes in cataract, particularly in human congenital cataract.

Methods Employed: The cultured lens epithelial cells were investigated using transmission and scanning electron microscopy. The lens cells were cloned using the microtest plate method, and the resulting cell lines were studied biochemically with slab gel electrophoresis and autoradiography. To determine the amount of the crystallins present in our cloned cell lines, the <sup>125</sup>I-radioimmunoassays for alpha, beta, and gamma crystallins were developed.

Major Findings: We have established a tissue culture approach to study congenital and hereditary cataracts. Progress in the understanding of congenital cataracts has been hampered by the lack of biological material. One possibility is to culture lens epithelial cells so that ample material will be available for biochemical analyses, which we hope would lead to understanding of the genetic defect. Since sufficient headway has been made in understanding the biochemical basis for the Nakano cataract, the lens epithelial cells from the Nakano mice provided us a way to test the feasibility of the tissue culture approach to study this type of cataract.

Mouse cells have been successfully cultured. The cells from both normal and Nakano mice have retained their epithelial nature even after one year in culture. One of the most unusual features of these cells is the presence of lentoid bodies. Some cells in the lentoid structure appear similar to the lens fiber cells in that the lack of cellular organelles creates a homogenous cytoplasm. Antibodies prepared against mouse  $\gamma$  crystallin react with the lentoid bodies suggesting that some cells in the lentoid structure produced crystallin. Since  $\gamma$  crystallin synthesis and loss of cellular organelles are properties of differentiated fiber cells, it appears that some cells in tissue culture retain the ability to express some differentiated characteristics. These cells have consistently shown these characteristics for over 1 year even with repeated subculture.

Another evidence that culturing mouse lens cells does not lead to loss of differentiated traits of the lens is the demonstration that the Nakano cells retain the Na-K ATPase inhibitor. This factor is thought to be responsible for electrolyte imbalance and increased hydration, the changes that precede cataract formation in the lenses of these animals. The inhibitor is found only in the cells from the lenses of the Nakano mice and not from the cells of the normal mice. Since the cells generally are subcultured without difficulty and the doubling time is relatively short, the purification of the inhibitor from these cells may be possible. The tissue culture studies with the Nakano lens cells suggest that biochemical defects can be uncovered by this approach. Thus it is hoped that culture of lens cells of human congenital cataracts may reveal the mechanism that initiates the cataractous process.

Further details of these cultured cells revealed that, as might be expected, the Nakano cells were less viable than the normal, although we have now

established both "cell" lines. When lens cell cultures from normal and Nakano mice were assayed, only the cultures from the cataract contained the transport ATPase inhibitor. The cell cultures which had high inhibitory activity were followed through successive passages. It was found that the inhibitory activity decreased after several passages. Apparently the uncloned cultures contained some cells with inhibitor and some without. Several passages of the cultures appeared to eliminate the cells containing the ATPase inhibitor.

Clonal analysis revealed another interesting feature. Cloning the cells with inhibitor through successive passages revealed that those cells retained the inhibitor and the level of inhibitor remained constant. Thus the cells appeared programmed not only to produce the inhibitor but to produce it in a certain quantity as well.

Another advance made was the development of the radioimmunoassay (RIA) for the lens crystallins. This method was essential to determine the small quantities of crystallins in the tissue-cultured cells. The RIA provided a sensitive method for quantitating  $\gamma$  crystallin in lens cell cultures from Nakano cataractous and normal mice. In addition, changes in lens crystallins could be followed during the aging of the Nakano and normal mice.

Purified  $\alpha$  and  $\gamma$  mouse lens crystallins were iodinated by the lactoperoxidase method. Both  $\alpha$  and  $\gamma$  crystallins maintained their immunological activity to their respective antisera as evidenced by a maximum binding of 75-80%. The most sensitive titer of antisera from rabbits that reacted at 50% of maximum binding was selected for the radioimmunoassay.

A standard inhibition curve against known quantities of crystallin served as the basis for quantitating unknown samples. The free counts were separated from the bound counts by precipitating the antigen-antibody complex with goat antiserum against rabbit gamma globulin. The sensitivity of the RIA was 2 ng. for  $\alpha$  crystallin and 4 ng. for  $\gamma$  crystallin.

With the RIA, it was possible to quantitate the amount of  $\gamma$  crystallin present in lens cell cultures. The results were consistent with the previous immunofluorescent observation that  $\gamma$  crystallin was localized in the lentoid bodies.

In aging of the normal and the cataractous lenses, the RIA revealed that a drastic reduction in  $\gamma$  crystallin occurred once the cataract was fully developed. In contrast, the percentage of  $\alpha$  crystallin in the soluble lens protein fraction remained unchanged during the period of aging studied.

#### Significance to Biomedical Research and the Program of the Institute:

One way to determine the possible metabolic changes in human cataracts, particularly congenital cataracts, is to study epithelial cells in a long-term, controlled situation. Tissue culture offers a defined environment in which to study interactions of various substances on the cells of the lens. With the congenital cataract, it may be possible to define the cause of the opacification of the lens. Tissue culture methods are one way to isolate rapidly a

particular property such as the Na-K ATPase inhibitor of a particular enzyme from lens cells in quantities sufficient for biochemical analyses.

The development of the radioimmunoassay aids in the quantitation of the changes occurring in the lens during cataract development. These assays also have application in studying the low level of crystallins which may lead to the elevation in intraocular pressure in some individuals with cataracts.

Proposed Course: Studies using two dimensional gel electrophoretic fingerprinting will be undertaken to look at protein production of the cells. The response of the cells to changes in the level of various ions such as Na and K will be investigated in this manner. Isolation of the Na-K ATPase inhibitor from the Nakano cell cultures will be attempted to understand its mode of action. Work will continue in the culture of human lens epithelium also in order to develop long-term culture conditions. A particular effort will be made to study enzymes in the glycolytic pathway of the human cells to determine differences between normal lens and lens with congenital cataracts.

The radioimmunoassay will be developed for human crystallins not only to use with cultured cells but also to quantitate changes in the crystallins in whole lens. The level of crystallins will be determined in normal as well as cataractous human lenses to investigate alterations occurring during opacification. In addition, the level of the crystallins in aqueous humor will be determined to correlate with changes in permeability of the lens during cataract development.

NEI Research Program: Cataract--The Normal Lens/Congenital, Metabolic, and Genetic Cataracts

Publications:

Kabasawa I, Fukui HN: Glycoproteins of the cattle lens plasma membranes. Jpn J Ophthalmol (in press).

Kabasawa I, Tusnematsu Y, Barber GW, Kinoshita JH: Low molecular weight proteins of the bovine lens. Exp Eye Res 24:437-448, 1977.

Horwitz J, Kabasawa I, Kinoshita JH: Conformation of gamma crystallin of the calf lens. Exp Eye Res 25:199-208, 1977.

Kabasawa I, Fukui HN: Fetal calf lens  $\gamma$  crystallin. Jpn J Ophthalmol (in press).

Fukui HN, Epstsin DL: The effect of parachloromercuribenzenesulfonate (PCMB) on lens cation transport. Ophthalmic Res (in press).

Zigler JS Jr, Sidbury JB Jr: Studies on lens proteins from the smooth dogfish: Evidence for evolutionary conservation in vertebrate  $\beta$ -crystallins. Ophthalmic Res 8:92-98, 1977.

Masters PM, Bada JL, Zigler JS Jr: Aspartic acid racemization in heavy molecular weight crystallins and water-insoluble protein from normal human lenses and cataracts. Proc Natl Acad Sci USA 75:1204-1208, 1978.

Zigler JS Jr: Age related changes in the polypeptide composition of  $\beta$ -crystallin from bovine lens. Exp Eye Res 26:537-546, 1978.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00069-01 LVR
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PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Immunological and Biological Properties of Ocular Components

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Igal Gery	Ph.D.	Visiting Scientist	LVR	NEI
Other:	Ann Sciambi	B.A.	Chemist	LVR	NEI
	Robert Nussenblatt	M.D.	Clinical Associate	CB	NEI
	David BenEzra	M.D., Ph.D.	Visiting Scientist	CB	NEI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Vision Research

SECTION

Section on Biochemistry

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

1.5

PROFESSIONAL:

1.0

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Biological and immunological properties of ocular components were examined. Retinal rod outer segments (ROS) were found to be highly inhibitory to lymphocyte cultures. These inhibitory effects were found to be mainly due to the oxidizing activity of the ROS preparations (perhaps by the release of free radicals), since the inhibition could be averted by anti-oxidants like vitamin E or 2-mercaptoethanol. Two components of ROS, retinol and docosahexaenoic acid, had similar inhibitory effects. ROS were not inhibitory to macrophage cultures but, rather, were readily phagocytized by these cells.

Rabbits immunized with rat lens extracts responded with a dissociated immune response: serum antibodies reacted mainly against the organ-specific proteins (crystallins) from various species, while the cellular immune response was directed mainly against the species-specific antigens.

Project Description:

Objectives: A significant group of eye diseases is assumed to be due to autoimmune responses. The ocular components often involved in these conditions are the retinal ROS and lens proteins. This project is aimed at examining various properties of these components, mainly with regard to their immunogenicity and biological effects on lymphocytes and other cells.

Methods Employed: Preparations of ocular components, isolation and culturing of lymphoid cells or cell lines, and immunization of experimental animals were carried out by commonly used procedures. The effects of ocular components or tested chemicals on cell cultures were determined according to changes in the incorporation of thymidine or uridine (= synthesis of DNA or RNA) in cultures incubated with or without mitogens. Phagocytosis by macrophages was assessed by microscopic observation. Humoral immune responses were analyzed and evaluated by gel precipitation, while cellular immune reactions were assayed according to the increase in DNA synthesis in lymphocytes cultured with the tested antigens.

Major Findings: Preparations of bovine retinal ROS inhibit the synthesis of DNA and RNA in lymphocyte cultures. Cultures of macrophages, keratocytes, or certain cell lines were less susceptible to the effects of ROS. Cytosol preparations of the retina had no inhibitory effects on lymphocyte cultures, whereas whole homogenates were moderately inhibitory. High levels of inhibition were also induced by homogenates or membranous preparations from bovine brain, but not by those from the kidney. Two components of ROS, i.e. retinol and docosahexaenoic acid (a polyunsaturated fatty acid), inhibit the lymphocyte cultures similarly to ROS. The effects of ROS and these components were averted in the presence of the antioxidants, alpha tocopherol (vitamin E) or 2-mercapto-ethanol. Thus, the activity of inhibitory oxidants is suggested.

ROS preparations, which were not toxic for macrophages, were readily phagocytized by monolayers of these cells. Furthermore, addition of macrophages to lymphocytes protected the latter cells from the inhibitory effects of ROS or their components, similarly to the protection by the anti-oxidants.

Rabbits immunized with rat lens extracts exhibited a unique dissociation between their humoral (antibody) and cellular immune responses. Sera from these rabbits reacted to extracts of lens from various species, including their own (autologous lens). Also, the intensity of the cross reactions resembled that of the reaction to the immunizing rat lens. The antibodies were directed only against lens proteins ( $\alpha$ ,  $\beta$  and  $\gamma$  crystallins) and no precipitation reactions were detected against other rat organs (= no species-specific antibodies). On the other hand, lymphocytes from these immunized rabbits reacted vigorously to the species-specific antigens, while showing little or no reaction to the organ-specific antigens of the autologous or other lenses.

Significance to Biomedical Research and the Program of the Institute: Our findings concerning the effects of ROS on lymphocyte cultures (a) support the notion that peroxidation of ocular tissues produces toxic compounds which may be involved in the etiology of some diseases of unknown origin (e.g. retinitis pigmentosa or lipofuscinosis), (b) underline the role of anti-oxidants (like

vitamin E) in protecting tissues from damaging oxidative processes, (c) provide a new sensitive biological assay for the level and activity of peroxidation products, and (d) provide new data concerning the susceptibility of the immune system to oxidizing agents and the essential protective role of anti-oxidants.

The phagocytosis of ROS by macrophages may be used for studying various new aspects of the shedding and removal of "used" ROS discs. The protective effects of macrophages against oxidizing agents reveals a heretofore unknown activity of these cells.

The finding of the unique dissociation between the humoral and cellular immune responses to the lens components should be useful for a better understanding of the immune response to the lens, mainly in various pathological conditions.

Proposed Course: The mode of action of ROS on lymphocytes will be further analyzed. Human cells from various patients will be tested in order to examine the possibility that in some conditions, the handling of peroxidation products is abnormal. The phagocytosis of ROS by macrophages will be further studied and compared to that by pigment epithelial cells. The effects of modulators of phagocytosis will be tested in these two cell types.

The immune response to different lens components will be further examined by using various other serological and cellular immune tests.

NEI Research Program: Retinal and Choroidal Diseases--Visual Cells and Pigment Epithelium; Cataract--The Normal Lens

Publications:

Nussenblatt R, Gery I, BenEzra D: Tissue specificity of ocular antigens, in Silverstein AM (ed): Proceedings of the Second International Symposium on the Immunology and Immunopathology of the Eye. Paris, Masson & Cie (in press).

Gery I, Davies P: Immunoregulatory products of macrophages, in Cohen S, Oppenheim JJ, Pick E (eds): Biology of Lymphokines. New York, Academic Press (in press).



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRANURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00135-06 LVR																						
PERIOD COVERED October 1, 1977 to September 30, 1978																								
TITLE OF PROJECT (80 characters or less)  Biochemical Structure of Retina and Pigment Epithelium in Health and Disease																								
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																								
<table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 30%;">Helen H. Hess</td> <td style="width: 10%;">M.D.</td> <td style="width: 40%;">Medical Officer (Research)</td> <td style="width: 10%;">LVR</td> <td style="width: 10%;">NEI</td> </tr> <tr> <td rowspan="3">Other:</td> <td>Donald R. Bergsma</td> <td>M.D.</td> <td>Senior Staff Ophthalmologist</td> <td>CB</td> <td>NEI</td> </tr> <tr> <td>Peter Gouras</td> <td>M.D.</td> <td>Head, Section on Neurophysiology</td> <td>LVR</td> <td>NEI</td> </tr> <tr> <td>Carl Hansen</td> <td>Ph.D.</td> <td>Geneticist</td> <td>VRB</td> <td>DRS</td> </tr> </table>			PI:	Helen H. Hess	M.D.	Medical Officer (Research)	LVR	NEI	Other:	Donald R. Bergsma	M.D.	Senior Staff Ophthalmologist	CB	NEI	Peter Gouras	M.D.	Head, Section on Neurophysiology	LVR	NEI	Carl Hansen	Ph.D.	Geneticist	VRB	DRS
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	Peter Gouras	M.D.	Head, Section on Neurophysiology	LVR	NEI																			
	Carl Hansen	Ph.D.	Geneticist	VRB	DRS																			
COOPERATING UNITS (if any) Veterinary Resources Branch, Division of Research Services; American Histolabs, Inc. (contract); G.E. Bunce, Ph.D., Virginia Polytechnic Institute and State University, Blacksburg, VA																								
LAB/BRANCH Laboratory of Vision Research																								
SECTION Section on Biochemistry																								
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20014																								
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SUMMARY OF WORK (200 words or less - underline keywords)  The broad aim of the project is to study the <u>biochemical composition</u> of <u>retina</u> , <u>pigment epithelium</u> , and <u>rod outer segments</u> in normal circumstances and in <u>retinal</u> and <u>choroidal diseases</u> of experimental or genetic origins. Topics of current interest are: (a) study of the concentration and distribution of <u>inorganic constituents</u> by <u>flameless atomic absorption</u> ; (b) localization and physiological function of <u>Ca</u> in retina, pigment epithelium and choroid; (c) possible involvement of <u>Ca</u> , <u>Zn</u> and <u>Cu</u> in retinal and choroidal diseases; and (d) study of <u>hybrids</u> of <u>RCS rats</u> and <u>spontaneously hypertensive (SH) rats</u> to determine whether the slow onset type of <u>retinal degeneration</u> seen in the latter is inherited at the <u>rdy</u> or another gene locus or is due to light damage in an albino animal.																								

Project Description:

Objectives: To study the biochemical composition of retinal photoreceptor, neuronal, glial, and pigment epithelial cells in health and disease, and to explore possibilities for prevention or therapy of retinal and/or choroidal disease when a biochemical abnormality has been identified; diseases in which pigment epithelium (PE) is involved are of particular interest.

Methods Employed: Retinas, isolated rod outer segments (ROS), and PE of frogs and rats are being analyzed. Samples of urine are being studied in human cases of retinal degeneration. Methods include flameless atomic absorption spectroscopy, microscopy, and a number of standard biochemical laboratory techniques.

Major Findings:

## I. Localization and concentration of Ca in retina and pigment epithelium:

Ca has been studied in isolated bullfrog ROS, light or dark adapted in vivo. A new centrifugation medium (colloidal silica with inert coating) has enabled separation at low speeds of highly pure intact ROS from pigment epithelial granules that contaminate ROS illuminated in vivo. These studies have further supported our finding that the content of Ca in ROS changes in light and dark, being higher in the light. We demonstrated previously that Ca is principally localized within the internalized disks in ROS, a site advantageous for a role in photoexcitation and/or dark/light adaptation. However, many cone outer segments lack disks; they have no appreciable amount of stored internal Ca, only free external Ca and no Ca demonstrable by the pyroantimonate technique used to show Ca in ROS disks.

Flameless atomic absorption assays of in vivo dark-adapted ROS isolated in low Ca medium had 0.6 mmol Ca/Kg wet wt. or 0.1 to 0.2 mol Ca/mol rhodopsin. This result on dark-adapted isolated ROS of bullfrogs has been confirmed by others. This is equivalent to 1 mmol Ca/liter ROS water, and since intradisk water is only about 10% of total ROS water, intradisk Ca concentration in darkness (at maximum rod sensitivity) could exceed that in extracellular fluid. The Ca transmitter hypothesis of Hagins and Yoshikami requires that one photon bleaching one Rh molecule in a bullfrog disk release 100-1000 Ca ions to close the leaky Na channels and hyperpolarize the external membrane. The disk, which has about  $2 \times 10^6$  Rh molecules, contained  $4 \times 10^5$  Ca atoms when fully dark-adapted, which is many fold the number required if Rh or a related molecule is an effective photon activated Ca shuttle. So far, various techniques used by numerous investigators for stimulating release of Ca from dark-adapted disks have not given the required yields. However, the proportion of Ca that is bound to lipid and protein and may require special conditions for release has not been established.

## II. Studies of trace elements:

(a) The 24-hour urinary excretion of trace metals in humans with hereditary

retinal degeneration: We have been investigating a report of Gahlot et al. (1976) that the amount of Cu excreted in a 24-hour urine specimen by patients with primary retinitis pigmentosa (RP) may be 6 times normal. In collaboration with Dr. D. Bergsma, we have studied a total of 11 individuals with retinal degeneration and 3 normals (2 of which were sampled on 2 occasions). The family diagnoses and number of members were: (A) Autosomal recessive RP Family #1 (5), Family #2 (3), and Family #3 (1); (B) Autosomal dominant RP (1); and (C) Usher's syndrome (1). Analyses were performed by flameless atomic absorption after treatment of urine with nitric acid, and the replicates checked within 3-5%. In the families with retinal degeneration, the results for Cu were all in the same range as the three normals and other normals reported in the recent literature (less than 30  $\mu\text{g}/24$  hours). A similar report of normal Cu in RP urine has been made by Ehlers and Bulow (1977). None of these studies, however, represents any large number of cases of different types of RP, so the possibility cannot be dismissed that a genetically special group may have been studied by Gahlot. For plasma, Cu values were reported by Ehlers and Bulow to be normal in their group of patients, but Bastek et al. (1977) found that in a group of sex-linked RP patients and carriers the mean plasma Cu was significantly higher than normal; plasma Zn was also found (Bastek et al.) to be higher in carriers than in RP victims or controls.

We determined Zn in the same urines in which Cu had been studied. Analyses were done by flameless atomic absorption after nitric acid treatment, followed by a 100 fold dilution, and replicate assays were within 3-5%. In the three normals, the values for Zn agreed with normals in the literature (below 700  $\mu\text{g}/24$  hours). In the autosomal recessive RP family #1, an abnormally high value was found (1092  $\mu\text{g}/24$  hours) in a son with moderately advanced RP; an unaffected brother had a value of 863, while a teen-aged sister with RP and the mother and father (carriers) had values below 700. In the autosomal recessive RP family #2, an abnormally high value (1715) was found in a 16-year-old son who had advanced RP but preserved central vision; the values for the mother and father (carriers) were between 700 and 800. A value below 700 was seen in the case of Usher's syndrome.

In summary, in the genetic types of RP studied so far, the 24-hour urine Cu was not elevated. Abnormally high 24-hour urinary Zn, however, was observed in two cases of autosomal recessive RP, and further investigation of urinary Zn in this type of RP seems needed.

(b) Trial of diets of different mineral composition for parturition and growth in RCS and congenic strains: Poor survival of young has been a constant problem in our biochemical studies with rats maintained on NIH standard rat lab chow. A mineral constituent that has been shown to be vital for reproduction and early growth of rats and improves performance in inbred strains in particular is zinc (consultation with Dr. G.E. Bunce). The open formula diet of NIH lab chow contains a minimal level of added Zn with a relatively high content of Ca and phytate, both of which could competitively inhibit Zn absorption. Accordingly, we have switched to Agway diets which contain a higher level of Zn. Our RCS rats have been consuming the new diets for three to four months, and both first litter and late litter females (older than one year) appear to produce larger numbers of pups which are of more uniform size and strength.

## III. Studies of hybrid rats from two strains with retinal degeneration:

As mentioned in the 1975 NEI Annual Report, Dr. Hansen, geneticist of the small animal section of the Veterinary Resources Branch of DRS, has been breeding the SH strain of rats developed from the Wistar strain at Kyoto University, Japan, as well as the RCS strain of rats that are homozygous for the *rdy* gene of retinal degeneration. We have been examining the retinal degeneration shown by the SH strain, which has a later onset and slower progression of the disease and may be a more realistic model for human RP than the RCS strain, as suggested by Mizuno et al. of Tohoku University, Sendai, Japan.  $F_1$  hybrids from a cross between the SH strain (albino) and the RCS strain (tan-hooded) have normal to low blood pressure, and last year we reported that at two years of age, 28 such hybrids (black-hooded) have normal rod photoreceptors, as shown by microscopy of both fresh and fixed stained specimens. Since retinal degeneration occurs at a few weeks of age in the RCS rat and by four to twelve months of age in the SH rat, the  $F_1$  hybrid (even though pigmented) would be expected to show changes by 12 months (and certainly by two years) if the *rdy* gene of the RCS were present in the SH genome, or if a different abnormal gene were present at the same locus.

From this study we concluded that the SH rat has no abnormal gene at the *rdy* locus. The possibility remained, however, that albino rats in which slow onset retinal degeneration has been described may have photoreceptor damage from light exposure. To help rule out this factor and to explore whether the SH rat may have an abnormal gene at a different locus,  $F_2$  animals ( $F_1 \times F_1$ ) were bred and tested at 18 and 22 months to determine the integrity of the electroretinogram (ERG). With collaboration of Dr. Peter Gouras (Section of Neurophysiology, NEI), the ERG's were recorded after overnight dark adaptation using a range of neutral density filters (4.0 to 0.0). The results showed a 25% frequency of extinguished ERG's, presumably homozygous *rdy*, in 6 of 23 rats. Of the remaining animals, 4 had B waves with amplitudes in the low range (below 500  $\mu$ V), while the others ranged up to a normal of 1000  $\mu$ V. Thus, a fourth (4/17) appeared to show a retinal degeneration factor from the SH strain, independent of the *rdy* gene. Mizuno et al. found that SH rats had low voltage responses or extinction by four to twelve months, while normal albinos had only slightly decreased amplitudes. The color ratios of our  $F_2$  rats were two black hoods to one tan hood to one albino, so that half had pigmented eyes. Among black-eyed animals, light damage does not occur.

Our finding that, beyond the expected 25% incidence of extinguished ERG's from the *rdy*, several animals had B wave amplitudes below 500  $\mu$ V is consistent with an independent effect of the SH genome in producing retinal degeneration, as suggested by Mizuno, Ozawa, Nishida and Aoki (1972) and Hayasaka, Takahashi and Mizuno (1977). Persistence of retinal degeneration in the SH strain over many generations of inbreeding suggests it is hereditary, but the nature of the primary gene defect in the SH rat has not been identified. The photoreceptor degeneration is not a consequence of the hypertensive retinopathy (arteriolo-sclerosis), since the outer half of the retina containing the photoreceptors is supplied by the choroid and is usually well-preserved, as it is in human hypertensive retinopathy. Instead, the inner retinal layers supplied by the retinal

arteries are damaged in the disease. Whether the SH retinal degeneration is intrinsically a manifestation of the hypertensive gene or gene complex or rather a part of a metabolic compensatory reaction remains to be ascertained.

Significance to Biomedical Research and the Program of the Institute:

Elucidation of the role or roles of Ca in photoreceptors could have broad significance for understanding retinal function in health and disease. Factors and control mechanisms for Ca movement and function in relation to photoreceptors need further study. They appear to be related to (1) changes in cyclic nucleotides during illumination, (2) the level of rod photosensitivity, (3) ROS tip shedding, and (4) pigment epithelial phagocytosis of ROS tips. Identification of a systemic metabolic abnormality in a readily available tissue or fluid, such as urine, in an RP patient at some interval in the disease or in some genetic type of the disease would provide a point of departure for more critical investigations. A diet increasing reproductive capacity of inbred strains of rat such as the RCS and congenic controls would greatly facilitate biochemical developmental studies requiring predictable numbers of healthy animals at successive postnatal days of age. Our work on RCS/SH hybrids indicates that the SH retina degeneration is most likely of a heritable type, independent of the rdy gene of the RCS, and supports the suggestion of Mizuno et al. that the SH may be a more appropriate model than the RCS for human RP.

Proposed Course: In vivo light/dark changes in Ca in ROS and pigment epithelium will be examined more critically in relation to ATP, cyclic nucleotides, Ca-binding proteins, and other possible factors. Relationships of Ca, Zn and other mineral constituents to immunological responses and phagocytosis in leucocytes and macrophages as well as pigment epithelial cells will be studied (in collaboration with Dr. I. Gery, immunologist). A rapid quantitative biochemical assay for phagocytic activity by fluorometric or chemiluminescence techniques will be adapted for this purpose. Cells from RCS and congenic controls will be utilized.

Additional 24-hour urine specimens from RP patients of autosomal recessive background will be assayed for Zn and Cu (collaboration with Clinical Branch, NEI). Nutritional observations of dietary factors in the RCS and congenic strains will continue with the objective of obtaining a maximum yield of healthy young for biochemical studies. Histopathological study will be done of the SH/RCS hybrid rats on which ERG's were carried out. Future work on SH rats will concentrate on pigmented animals which can be developed by Dr. Hansen. This will eliminate any possible effects of light damage and create a more suitable model of the human pigmented eye.

NEI Research Program: Retinal and Choroidal Diseases--Visual Cells and Pigment Epithelium

Publications:

Hess HH, Lees MB, Derr JE: A linear Lowry-Folin assay for both water-soluble and sodium dodecyl sulfate-solubilized proteins. Anal Biochem 85:295-300, 1978.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00007-04 LVR																		
PERIOD COVERED October 1, 1977 to September 30, 1978																				
TITLE OF PROJECT (80 characters or less)  The Biochemical Pharmacology of the Eye																				
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																				
<table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 25%;">Hitoshi Shichi</td> <td style="width: 15%;">Ph.D.</td> <td style="width: 20%;">Research Chemist</td> <td style="width: 10%;">LVR</td> <td style="width: 15%;">NEI</td> </tr> <tr> <td>Other:</td> <td>Noveen D. Das</td> <td>Ph.D.</td> <td>Postdoctoral</td> <td>LVR</td> <td>NEI</td> </tr> <tr> <td></td> <td>Daniel W. Nebert</td> <td>M.D.</td> <td>Chief</td> <td>DPB</td> <td>NICHD</td> </tr> </table>			PI:	Hitoshi Shichi	Ph.D.	Research Chemist	LVR	NEI	Other:	Noveen D. Das	Ph.D.	Postdoctoral	LVR	NEI		Daniel W. Nebert	M.D.	Chief	DPB	NICHD
PI:	Hitoshi Shichi	Ph.D.	Research Chemist	LVR	NEI															
Other:	Noveen D. Das	Ph.D.	Postdoctoral	LVR	NEI															
	Daniel W. Nebert	M.D.	Chief	DPB	NICHD															
COOPERATING UNITS (if any) National Institute of Child Health and Human Development																				
LAB/BRANCH Laboratory of Vision Research																				
SECTION Section on Biochemistry																				
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20014																				
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:																		
0.5	0.5	0.0																		
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																				
SUMMARY OF WORK (200 words or less - underline keywords)																				
<p>           Following an intraperitoneal injection of <u>acetaminophen</u> into polycyclic hydro-            carbon-responsive mice in which hepatic <u>aryl hydrocarbon hydroxylase</u> (Ah)            activity had been induced by pretreatment with polycyclic hydrocarbons,  <u>lenticular opacification</u> developed in a few hours. Both Ah/Ah<sup>+</sup> <u>homozygous</u>            and Ah/Ah<sup>-</sup> <u>heterozygous</u> inbred mouse strains developed the lenticular opacity.            The <u>ciliary body of the eye</u> of polycyclic hydrocarbon-responsive mice was            found to possess high activities of Ah and <u>γ-glutamyl transpeptidase</u> (an            enzyme involved in <u>mercapturic acid formation</u>). Therefore, the ciliary body            might detoxify hydrocarbons and drugs in the blood at the time of <u>aqueous humor</u>  <u>secretion</u>.         </p>																				

Project Description:

Objectives: We have previously demonstrated that aryl hydrocarbon hydroxylase (AHH) induction in the eye (pigmented epithelium) and in the liver of polycyclic hydrocarbon-responsive mouse strains is apparently under the same genetic regulation.

In this work we report that administration of large doses of acetaminophen to mice in which AHH has been induced by pretreatment with polycyclic hydrocarbons (e.g. 3-methylcholanthrene) causes development of lenticular opacification in a few hours. A possible mechanism of the lens opacity formation is investigated. We also report the existence of drug-metabolizing activities in the ciliary body of the eye.

Methods Employed: Mice were injected intraperitoneally with 3-methylcholanthrene to induce AHH activity for 48 hours. Acetaminophen was then injected intraperitoneally into pretreated mice, and development of lenticular opacification was examined in vivo as well as in vitro. Eyes were fixed and subjected to histochemical examination. Glutathione levels of lens and liver were determined by a spectroscopic method. Covalent binding of acetaminophen metabolites to lens, liver, and other tissues was studied with <sup>3</sup>H-acetaminophen. Gamma glutamyl transpeptidase in the bovine ciliary body was assayed spectroscopically with  $\gamma$ -glutamyl P-nitrophenol.

Major Findings: The aryl hydrocarbon hydroxylase (Ah) locus can be correlated with the differences in responsiveness to polycyclic and halogenated aromatic compounds between C57BL/6 (responsive Ah<sup>+</sup>) and DBA/2 (nonresponsive Ah<sup>-</sup>) inbred strains. Heterozygotes (Ah<sup>+</sup>/Ah<sup>-</sup>) are responders.

In the present work we established a positive correlation between the Ah<sup>+</sup> allele and acetaminophen-caused cataract. We found that acetaminophen causes cataracts in other methylcholanthrene-treated responsive inbred strains such as A/J, CBA/J, and C3H/HeJ but not in other methylcholanthrene-treated nonresponsive inbred strains such as RF/J, AKR/J, SJL/J, and SWR/J. The mechanism of acetaminophen<sub>3</sub>-induced cataract formation is uncertain. However, reactive intermediates of <sup>3</sup>H-labelled acetaminophen were shown to bind to the lenses of the genetically responsive mice. Involvement of acetaminophen metabolites was also suggested by the fact that prior treatment with phenobarbital, which enhances glucuroride conjugation of acetaminophen in the liver and is independent of the Ah locus, prevented cataracts in the responsive mice. Reactive intermediates of acetaminophen formed predominantly in the liver but are also formed in numerous nonhepatic tissues. We found that the ocular ciliary body of responsive mice possesses high activities of Ah and  $\gamma$ -glutamyl transpeptidase (an enzyme involved in mercapturic acid formation). Therefore, some of the acetaminophen reaching the tissue in blood circulation could be activated (and/or detoxified) before it is secreted into aqueous humor.

Significance to Biomedical Research and the Program of the Institute: The present study on lenticular opacification caused by acetaminophen in mice shows that AHH-inducible strains are particularly susceptible to the toxic effect of

acetaminophen on the lens. The present results on experimental animals, however, should not be extrapolated to humans until further studies are made.

Proposed Course: This project will be continued.

NEI Research Program: Retinal and Choroidal Diseases--Visual Cells and Pigment Epithelium/Special Areas of Future Interest (Toxic and Environmental Disorders); Cataract--Cataract Induced by Drugs and Radiation and Secondary to Other Eye Disorders

Publications:

Shichi H, Gaasterland DE, Jensen NM, Nebert DW: Ah Locus: Genetic differences in susceptibility to cataracts induced by acetaminophen. Science 20:539-541, 1978.

Shichi H, Kumaki K, Nebert DW: Circular dichroism studies on the binding of type I substrates and reverse type I compounds to rabbit liver microsomal cytochrome P450. Chem Biol Interac 20:133-148, 1978.

Shichi H, Nebert DW: Drug metabolism in ocular tissues in Extrahepatic Metabolism of Drugs and Other Foreign Compounds, Gram TE (ed). Spectrum Publications, Inc. Jamaica, NY (in press).



## PERIOD COVERED

October 1, 1977 to September 30, 1978

## TITLE OF PROJECT (80 characters or less)

The Biochemistry of the Visual Process

## NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Hitoshi Shichi	Ph.D.	Research Chemist	LVR	NEI
	Alois J. Adams	Ph.D.	Postdoctoral Fellow	LVR	NEI
Other:	Robert L. Somers	B.S.	Chemist	LVR	NEI
	Consuelo G. Muellenberg	B.A.	Biologist	LVR	NEI

## COOPERATING UNITS (if any)

None

## LAB/BRANCH

Laboratory of Vision Research

## SECTION

Section on Biochemistry

## INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20014

## TOTAL MANYEARS:

3.5

## PROFESSIONAL:

1.5

## OTHER:

2.0

## CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS☐ (b) HUMAN TISSUES☒ (c) NEITHER☐ (a1) MINORS ☐ (a2) INTERVIEWS

## SUMMARY OF WORK (200 words or less - underline keywords)

1) Rhodopsin kinase that catalyzes the phosphorylation of rhodopsin with ATP has been purified to a homogeneous state. Properties of the kinase and the intracellular localization of the phosphorylation reaction have been investigated.

2) The location of the carbohydrate moiety of rhodopsin in the retinal disk membrane has been shown to be exclusively on the inner surface of the membrane. On the other hand, the phosphorylation sites of rhodopsin are located on the outer surface of the disk membrane. These results indicate that rhodopsin is a transmembrane protein.

Project Description:

Objectives: The overall objectives of this project are to investigate the light-dark adaptation processes of the retina by means of modern techniques of biochemistry and membrane biology. More specifically, these are (1) identification of a sequence of molecular events initiated by absorption of photons and leading to visual transduction (light process) and (2) elucidation of the biochemical mechanism of regeneration of the photosensitivity of photoreceptor membranes (dark process). The investigations presented in this report deal with two aspects of the visual pigment rhodopsin, i.e. (a) phosphorylation of rhodopsin by a rod outer segment protein kinase, and (b) concanavalin A binding and orientation of rhodopsin in the rod disk membrane.

Methods Employed: Biochemical methods such as centrifugation, column chromatography, spectroscopic analysis, and radioisotope assay.

Major Findings: (1) Phosphorylation of rhodopsin

Rhodopsin kinase was extracted from bovine rod outer segments with 1 M ammonium chloride and purified by ammonium sulfate fractionation and chromatography on Sephacryl S-200 and Blue Sepharose CL6B. The purified kinase was found to be essentially homogeneous in polyacrylamide gel electrophoresis with 0.1% sodium dodecyl sulfate. The molecular weight of kinase was estimated to be 50,000-53,000 daltons from the electrophoretic mobility and by gel filtration on a calibrated Sephacryl column. There is no evidence that the enzyme is composed of subunits. The enzyme was specific for rhodopsin; phosphotyrosine, casein, histone, and protamine were not phosphorylated. Both ATP and GTP were utilized but ATP ( $K_m = 8 \mu\text{M}$ ,  $V_{max} = 40 \text{ nmol/mg} \cdot \text{min}$ ) was the preferred substrate to GTP ( $K_m = 400 \mu\text{M}$ ,  $V_{max} = 2 \text{ nmol/mg} \cdot \text{min}$ ). The activity was inhibited 90% by 1 mM  $\text{Zn}^{+2}$ , 50% by 1 mM AMP and 50% by 1 mM adenosine but not by cyclic AMP or cyclic GMP.  $\text{Na}^+$  (100 mM) was a potent inhibitor (90% inhibition) of the enzyme, while  $\text{K}^+$  (100 mM) was without effect. In order to identify the phosphorylated protein in native form, urea-treated rod outer segments were phosphorylated in the light with purified kinase and ATP, and incubated with retinal in the dark to regenerate the visual pigment. Phosphorylated protein was extracted and purified on ECTEOLA-cellulose. Phosphorylated pigment (about 16% of the total pigment) which was completely separated from unphosphorylated pigment and spectrally characterized contained about 5 moles phosphate per mole pigment. It was thus concluded that the substrate for the light-dependent phosphorylation of rod membranes is a small fraction of rhodopsin that possesses multi-phosphorylation sites. To investigate the cellular location of the phosphorylation reaction, rod outer segments were prepared from living frogs in which newly formed disks had been labelled with [ $^3\text{H}$ ]-leucine, and phosphorylated. The pigment was extracted and purified as above. The [ $^3\text{H}$ ]-radioactivity per pigment was significantly higher in phosphorylated pigment than in unphosphorylated pigment. The result led us to conclude that newly formed disks (hence, the plasma membrane as well, that is continuous with the disk infolding) are preferentially phosphorylated.

(2) Concanavalin A binding and orientation of rhodopsin in the disk membrane.

Centrifugation of bovine rod outer segments in a continuous metrizamide [2-(3-acetamido-5-N-methylacetamido-2,4,6-trifiodobenzamido)-2-deoxy-D-glucose] gradient resulted in the separation of two outer segment bands. The lower (higher-density) band was stained very extensively with the fluorescent marker didansyl-L-cystine, but the upper (lower-density) band was stained only weakly. This was interpreted to indicate that the outer segments in the upper band are closed, while those in the lower band are open or leaky. The closed outer segments were found to bind to concanavalin A Sepharose beads. The binding was inhibited by  $\alpha$ -methylmannoside. The disks were released from closed outer segments by incubation in 5% Ficoll and chromatographed on a concanavalin A Sepharose column. Essentially all disks were collected in the void volume of the column; less than 10% of the disks loaded were eluted with  $\alpha$ -methylmannoside. If the disks were freeze-thawed and placed on the column, however, a considerable fraction of the disks became capable of binding to the column. Uptake of radioactive insulin by the disks occurred during freezing, indicating an inversion of the disks. The results led us to conclude that the concanavalin A binding sites of intact disks are located on the internal surface and are exposed by freeze-thawing. This was confirmed by the binding of concanavalin A-ferritin to freeze-thawed disks but not to intact disks. Photoc bleaching of rhodopsin did not alter the binding properties of outer segment and disks. By labelling rhodopsin in the inverted disk membrane with radioactive UDP galactose and galactosyl transferase and purifying the labelled pigment, the carbohydrate moiety of rhodopsin was shown to be involved in concanavalin A binding by the disk membrane. Using purified kinase and ATP, rhodopsin in the intact and inverted disks was phosphorylated. Extensive phosphorylation of rhodopsin occurred only in intact disks. From this finding and the asymmetric distribution of the concanavalin A binding sites of rhodopsin we concluded that rhodopsin is a transmembrane protein with the phosphorylation sites on the outer surface and the concanavalin A binding sites on the inner surface.

Significance to Biomedical Research and the Program of the Institute: The present results that rhodopsin phosphorylation takes place primarily in the newly formed disks and the plasma membrane of the rod suggest a possible involvement of the reaction in disk formation (i.e. membrane assembly). Alternatively, the reaction may be related to the biochemical mechanism of dark adaptation of the newly formed disks. In any case, the reaction undoubtedly plays an important role in the photoreceptor function.

Proposed Course: This project will be continued.

NEI Research Program: Retinal and Choroidal Diseases--Visual Cells and Pigment Epithelium

Publications:

Shichi H, Kawamura S, Muellenberg CG, Yoshizawa T: Isochromic forms of rhodopsin: Isolation and photochemical properties. Biochemistry 16: 5376-5381, 1977.

Shichi H, Somers RL: Light-dependent phosphorylation of rhodopsin: Purification and properties of rhodopsin kinase. J Biol Chem (in press).

Adams AJ, Tanaka M, Shichi H: Concanavalin A binding to rod outer segment membranes: Usefulness for preparation of intact disks. Exp Eye Res (in press).

Adams AJ, Somers RL, Shichi H: Orientation of rhodopsin in the disk membrane. Photochem Photobiol (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00138-06 LVR
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PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

The Visual Cell: Process of Photoexcitation and Restoration

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. Yoshikami	Ph.D.	Research Biologist	LVR	NEI
Other:	G.N. Noll	Ph.D.	Visiting Associate	LVR	NEI
	C. Albani	M.D.	Guest Scientist	LVR	NEI
	M. Yoshida	Ph.D.	Guest Scientist	LVR	NEI
	W.A. Hagins	M.D., Ph.D.	Chief, Section on Membrane Biophysics	LCP	NIAMDD

COOPERATING UNITS (if any)

Laboratory of Chemical Physics, NIAMDD

LAB/BRANCH

Laboratory of Vision Research

SECTION

Section on Biochemistry

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

3.0

PROFESSIONAL:

3.0

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS      ☐ (b) HUMAN TISSUES      ☒ (c) NEITHER

☐ (a1) MINORS    ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A definitive test for our hypothesis that calcium is a principal agent in the initiation of vision is the measurement of transient light-stimulated changes in calcium ion activity in the light-receiving visual cell, and for this purpose we investigated the properties of a calcium sensitive dye dichlorophosphonazo-III (DCP3) and methods of introducing it into cells.

A major problem facing cell biologists is one of introducing into live cells for a variety of purposes large, electrically charged molecules such as DCP3 which are excluded by the cell membrane. We have developed a method to overcome this in part which now allows us to introduce DCP3 into visual cells.

On the other hand, lipid soluble materials have no difficulty entering cells, but their insolubility and instability in physiological solutions hamper their introduction into cells in culture or perfusion. We have used phospholipid vesicles to deliver to live cells, water insoluble, oxygen sensitive compounds like retinol. In this manner visual pigment can be restored in isolated retinas. The process of the restoration of visual pigment and its relation to the photoelectrical activity of the visual cell are now being studied.

Project Description:

Objectives: To study the nature of the visual cell and determine its physical and chemical means of initiating and sustaining the phenomenon of vision.

Methods Employed: The delicacy, small size, and other properties of the visual cells force novel approaches to their study. A concerted application of a combination of chemical and physical methods involving electrical, biochemical, optical, and anatomical measurements are used to study the retina and its associated ocular tissues.

Major Findings: Dichlorophosphonazo III (DCP3) absorption spectra and its affinity to ions of physiological importance were measured (Yoshikami & Hagins) and were found to be suitable for our study. It has a  $K_D = 10^{-6} M$  at pH 7.0 and was found to have distinct spectral signatures for  $Ca^{++}$ ,  $Mg^{++}$ ,  $K^+$ ,  $Na^+$ , and  $H^+$  whose activity change can be resolved by our rapid kinetic multi-wavelength spectrometer.

DCP3 is a large anion that is impermeable to cell membranes. The phospholipid vesicle method of introducing membrane impermeable substances does not introduce a sufficient quantity of dye fast enough for our needs. We have sought alternative methods, studied the general problem of transferring large, charged water soluble molecules across plasma membrane of live cells, and have developed a technique to introduce them into cells. Studies are now being done on introducing dyes into the live retina to test the calcium hypothesis for photoexcitation.

Gecko retinas are unusual in many respects, one of which is the blue shift of the absorption spectrum shown by their detergent-extracted visual pigment when the chloride activity in the detergent solution is changed. We (Yoshida, Noll, and Yoshikami) have investigated whether this change also occurs with the visual pigment in the living retina and whether this shift in absorption spectra would affect the photoexcitability property of the retina.

When isethionate anion was transiently substituted for chloride in the retinal perfusate and it caused the visual pigment in the live Gecko retina to shift correspondingly its absorption spectrum maximum by 5 nm. The shift was found to be substantially less than what Crescitelli discovered to be in the detergent-extracted visual pigment. Further study is in progress.

The isolated and perfused retinas can maintain their photoresponses for extended times, but cannot restore any significant amount of visual pigment following a complete bleach. We (Yoshikami & Noll) have developed a technique of using phospholipid vesicles (PLV) to introduce water insoluble lipids into live cells, and with this method it is now possible to introduce to the living retina different kinds of retinol congeners to study the regeneration of visual pigment and assay the role of each retinol intermediate in the visual cycle. The PLV containing various congeners of retinol permit us to open the loop in the visual cycle and determine where and what happens to the itinerant retinol

congener as it courses between the pigment epithelium and the retina during the light and dark visual adaptation processes. We have shown that the transfer of lipid soluble materials unlike the transfer of water soluble compound by the PLV, does not necessitate vesicle fusion or ingestion of them by the cell.

The visual cycle in amphibian retina (frog) was found to differ significantly from that in mammalian retinas (rat, guinea pig, and cattle). The amphibian retina alone is able to oxidize 11-cis retinol to its corresponding aldehyde to make visual pigment, but the isolated mammalian retinas are unable to do so; they can regenerate visual pigment only if 11-cis retinaldehyde is supplied to them.

The plasma membrane encloses but is not connected to the disc membrane surfaces. If there were rhodopsin in the plasma membrane as there is in the disc membranes and if the 11-cis retinaldehyde were supplied to a retina from an extracellular source like the PLV as it would be in the case with isolated retinas, then one should be able to observe two rates of rhodopsin regeneration in the retinal rod cell where the rate in the plasma membrane should be higher than that in the disc membrane. Furthermore, since the amplitude of the fast photovoltage (FPV) is linearly related to the amount of rhodopsin in the retina, then the rhodopsin in the plasma membrane would be the source of the FPV because of the membrane structural arrangement, and the relative recovery of the FPV amplitude in the bleached retina would thus indicate the level of rhodopsin regenerated in the plasma membrane. The FPV should be found to recover in a bleached retina ahead of the rhodopsin in the disc.

In the bleached isolated rat retina, given 11-cis retinaldehyde with the aid of PLV, we find the FPV amplitude to recover to 50% of its original value in 7.5 minutes and 100% by 15 minutes, but we find the rhodopsin in the disc to regenerate in 20 and 90 minutes to the respective values. These experiments show that rhodopsin is definitely present in the plasma membrane of the retinal rod cells and it is this population of rhodopsin that is responsible for the fast photovoltage.

Since the mammalian retina cannot isomerize all-trans retinol congeners to the 11-cis or use 11-cis retinol to form visual pigment, could the retinol congener returning to the retina from the pigment epithelium during dark adaptation be 11-cis retinaldehyde? If this were so, the returning retinol species would not have to be taken internally by the retina and converted before it could regenerate rhodopsin, and this routing would be reflected as a difference in the rates of rhodopsin regeneration in the plasma membrane and in the disc membrane of the eye of the living animal. The regeneration rates would appear similar to the isolated retina receiving 11-cis retinaldehyde from an external source.

In the bleached retina of the anesthetized rat, the rhodopsin in the plasma membrane, as assayed by the FPV, recovers to 50% of its original dark adapted value in 11 minutes, whereas the rhodopsin in the internal disc membranes took 40 minutes. This, taken together with other findings on the visual cycle of the mammalian eye, indicates it is the 11-cis retinaldehyde that returns to the retina from the pigment epithelium during dark adaptation, and so the reisomerization to 11-cis retinaldehyde from the all trans form in the mammalian eye must occur in the pigment epithelium cells.

Significance to Biomedical Research and the Program of the Institute: Our understanding of the causes and our ability to prevent and treat numerous visual disorders depend on a clear knowledge of the processes operant in normal vision. Our finding of the importance of calcium and its control in the visual cell excitatory process, and the revelation of the tight coupling between photoexcitation and energy metabolism of this cell, may help us to realize some of the basis for pathology in the retina. The concatenated reactions of retinol in two adjacent tissues, the pigment epithelium and retina, show these tissues are interdependent. The understanding of how retinol and other metabolites pass through the aqueous space between them has bearing on the vitality of both tissues, in particular where retinal detachment occurs.

The method of introducing into cells dyes capable of reporting ionic activities and the measurement of intracellular ionic activities should be useful in many other areas of biomedical research. The use of phospholipid vesicle as a carrier of water insoluble as well as water soluble membrane-impermeable substance to live tissues has broad ramifications which extend from pharmacology to genetic engineering and should be of general interest and importance.

Proposed Course: How the retina initiates and sustains vision is the focus points of our studies. We will continue to study the physical and chemical processes involved and pay particular attention to the support tissue like the pigment epithelium.

NEI Research Program: Retinal and Choroidal Disorders--Visual Cells and Pigment Epithelium

Publications:

Hagins WA, Yoshikami S: Intracellular transmission of visual excitation in photoreceptors: Electrical effects of chelating agents introduced into rods by vesicle fusion, in Fatt P, Barlow HB (eds): Vertebrate Photoreceptors. New York, Academic Press, 1977, pp 97-139.

Yoshikami S, Hagins WA: Calcium in excitation of vertebrate rods and cones: Retinal efflux of calcium studied with dichlorophosphonazo III. NY Acad Sci 307:545-561, 1978.

Yoshikami S, Noll GN: Isolated retinas synthesize visual pigment from retinol congeners delivered by liposomes. Science 200:1393-1395, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00036-02 LVR
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PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Development of the Chick Conjunctival Periderm and Conjunctival Papillae

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Ellen Porzig	Ph.D.	Staff Fellow	LVR NEI
Other:	Alfred J. Coulombre	Ph.D.	Head, Section on Experimental Embryology	LVR NEI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Vision Research

SECTION

Section on Experimental Embryology

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

1.5

PROFESSIONAL:

1.5

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The conjunctival papillae of the chick embryo are under study to determine the roles played by the peridermal cells in the development of the underlying perilimbic sclera. Specifically, we seek to describe the timetable and regional distribution of changes in the size, shape and surface characteristics of the peridermal cells and to correlate these changes with the development of the conjunctival papillae and of the scleral ossicles which arise under the influence of the papillae.

Project Description:

Objectives: The ectoderm, which covers the surface of vertebrate embryo is a stratified squamous epithelium comprising two layers, an inner basal layer of cuboidal cells and an outer layer of squamous cells called the periderm. While the basal layer has been shown to serve a number of important developmental functions, no functions have been demonstrated for the periderm. A favorable opportunity for identifying such functions is presented in the chick embryo by transient thickenings (papillae) in the conjunctiva. These structures appear on the seventh and eighth day of embryonic development as 14 focal thickenings of the conjunctiva in a ring surrounding the corneal limbus. The papillae disappear on the thirteenth day. During its brief existence, it is responsible for the induction of a bone (scleral ossicle) in the underlying mesenchyme.

This study focuses on the conjunctival periderm and seeks to answer the following questions. Are there changes in the periderm which correlate spatially and temporally with the development of the conjunctival papillae or the pattern of development in the ring of bones induced by the papillae in the underlying mesenchyme? Specifically, do changes in the shape, size, surface characteristics or mutual attachments of the peridermal cells occur at time when or in places where the papillae are active inductively? When, during development, does the conjunctival periderm desquamate? Does desquamation occur at the same time in all regions of the periderm, or are there consistent regional differences in the time of onset of desquamation? If such regional differences exist, how do they correlate with the development and involution of the papillae?

Methods Employed: The size and shape of the conjunctival peridermal cells will be determined over a range of ages in the chick embryo in specimens stained for cell outline by a modified silver nitrate impregnation. Photomicrographs and camera lucida tracings will be used for geometric and planimetric measurements of cell size and shape. The use of scanning electron microscopy in this project was abandoned during the year. A microsurgical procedure (previously developed by this Section) was used to remove individual papillae. During subsequent development the membrane bone, which normally would have appeared beneath such papillae, failed to develop and the gap thus created in the bony ring was subsequently filled by ingrowth of the two neighboring ossicles. In each of four different groups of seven-day-old chick embryos a single papilla was ablated (papilla numbers 4,6,11 or 13). At 14 days of incubation, bones adjoining these locations were isolated, measured for total area as well as for area ossified and compared with their counterparts from the opposite (control) eye.

Major Findings: In addition to the findings reported in FY77, the following points were established this year: (1) Ossicles which grow into a gap in the bony scleral ossicular ring become larger in area than their contralateral counterparts which develop in untreated rings; (2) The zones of ossification of such bones (assessed at 14 days of incubation) are also larger than the counterparts in the contralateral, untreated eye; (3) The ossification centers

in bone membranes growing into experimentally created gaps in the scleral ossicular ring did not extend to the free margins of the preosseous membrane. Thus, the overlap between adjacent preosseous membranes is not a factor in the localization of the ossification center; (4) A scleral preossicular membrane can grow into an experimentally produced adjacent gap from borders destined, in the normal course of development, either to overlap or to be overlapped by their nearest neighbor bones.

Significance to Biomedical Research and the Program of the Institute:

Just as the embryonic corneal epithelium has been shown to dictate the three-dimensional architecture of the stromal tissue underlying it, evidence is now emerging that the embryonic conjunctiva induces tissues in the mesenchyme beneath it and determines their structure. The sharp transition between corneal stroma and the limbic sclera appears to be attributable in large measure to the activities early in development of the quite different epithelia which overlie these regions. This study uses the favorable context of the chick embryonic conjunctiva and perilimbal sclera to explore some of the developmental roles of the conjunctiva, with special emphasis on the roles possibly played by its periderm. It is hoped that these efforts will clarify some aspects of normal and abnormal shaping of the anterior segment of the eye during embryonic development.

Proposed Course: This project will be continued in an attempt to reach its objectives.

NEI Research Program: Retinal and Choroidal Diseases--Myopia

Publications: None



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00177-03 LVR
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PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Plasma Membrane Composition and Biosynthesis in Chick Lens Fibers and Epithelia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Peggy Zelenka Ph.D. Geneticist LVR NEI

Other: None

COOPERATING UNITS (if any)

Joram Piatigorsky, Ph.D., Head, Section on Cellular Differentiation, Laboratory of Molecular Genetics, NICHD

LAB/BRANCH

Laboratory of Vision Research

SECTION

Section on Experimental Embryology

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

1.04

PROFESSIONAL:

1.04

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project seeks to determine whether the regulation of lens fiber differentiation and maturation is associated with alterations in the plasma membrane. To this end, the principal lipid and protein components of embryonic and adult chicken lens membranes are being identified, and their metabolism is being investigated. Because of the known involvement of phosphatidylinositol turnover in regulatory mechanisms of various other cell types, the initial stages of this study have focused on lens phospholipid metabolism. Computer modeling of the kinetics of <sup>32</sup>P incorporation into lens phospholipids in vivo is employed to determine the rates of synthesis and degradation of individual phospholipids. This approach can also be applied to the study of phospholipid metabolism in differentiating cultured explants of embryonic chick lens epithelia, thus allowing the possible relationships between phospholipid metabolism and differentiation to be studied under controlled conditions. Studies of lens membrane proteins are being conducted by applying standard techniques of protein chemistry to purified lens membranes.

Project Description:

Objectives: The objectives of this project are: a) to characterize the principal lipid and protein components of plasma membranes from embryonic chick lens fibers and lens epithelial cells; b) to determine whether the differentiation of lens epithelial cells into lens fibers is accompanied by changes in membrane composition; c) to learn whether the differentiation of lens epithelial cells into lens fibers is accompanied by changes in the metabolism of lens plasma membranes; and d) to establish the functional significance of any change in membrane composition or metabolism.

Methods Employed:  $^{32}\text{P}$ -labeled phospholipids are obtained by injecting isotope into six-day-old chick embryos via the chorioallantoic circulation. Lens fibers and epithelia are isolated by microdissection of the embryos. Phospholipids are extracted and separated by thin layer chromatography; radioactivity is determined either by scintillation counting or by autoradiography. Computer modeling is employed to determine rates of synthesis and degradation of individual phospholipids from the  $^{32}\text{P}$  incorporation data.

For studies of lens membrane proteins, lenses are obtained from chickens at various stages of development and maturation, and the lens membranes are purified by a combination of sucrose gradient centrifugation and centrifugation in urea. The membrane proteins are separated by SDS-polyacrylamide gel electrophoresis. Individual protein bands are iodinated with  $^{125}\text{I}$  and digested with trypsin; the tryptic peptides are separated by electrophoresis on thin layer plates and located by autoradiography.

Major Findings: The rates of synthesis per cell of all the major phospholipids of the six-day-old chick embryo lens (phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, and phosphatidic acid) are greater in the lens fibers than in the lens epithelial cells. Thus, an increase in the rates of synthesis of these phospholipids is associated with lens fiber differentiation. In addition, the rate of degradation of phosphatidylinositol per cell is less in the lens fibers than in the lens epithelial cells. Regulation of the rapid metabolism of this phospholipid, therefore, also is associated with the initial stages of lens fiber differentiation.

An intrinsic membrane protein has been identified in purified lens membranes which has the same subunit molecular weight as delta-crystallin, although its tryptic peptides are entirely different. This membrane protein is the only protein of subunit molecular weight 50,000 in membranes of the lens cortex of adult chickens; these membranes contain no delta-crystallin. Membranes of the lens nucleus of adult chickens, as well as embryonic lens membranes, seem to contain both the membrane protein and delta-crystallin.

Significance to Biomedical Research and the Program of the Institute: The plasma membranes of lens cells appear to play important roles in normal development and function of the lens. In addition, they are centrally involved in the genesis and development of several varieties of lens cataract. Despite the widely recognized and important functions of these membranes, work on their

composition, turnover, and development has begun only recently. This project focuses on changes in lens cell membranes which are associated with lens fiber differentiation. These results should have broad application in understanding normal lens differentiation and morphogenesis and in attempts to establish etiologies for several types of cataract.

Proposed Course: This project will be continued. An attempt will be made to study lens phospholipid metabolism in cultured explants of embryonic chick lens epithelia during in vitro lens fiber differentiation. The effects of various agents which promote or prevent differentiation will be investigated. Techniques will be developed to study the metabolism of the polyphosphoinositides in the cultured lens epithelial explants.

Additional experiments will be conducted to determine whether delta-crystallin is an intrinsic membrane component of embryonic chick lenses and to determine whether delta-crystallin and the membrane protein that has the same subunit molecular weight are immunologically related.

NEI Research Program: Cataract--The Normal Lens

Publications:

Zelenka P: Phospholipid composition and metabolism in the embryonic chick lens. Exp Eye Res 26:267-274, 1978.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE <b>NOTICE OF          INTRAMURAL RESEARCH PROJECT</b>	PROJECT NUMBER  Z01 EY 00032-02 LVR																								
PERIOD COVERED October 1, 1977 to September 30, 1978																										
TITLE OF PROJECT (80 characters or less)  Effects of Vitamin A Deficiency on the Neural Retina and Cornea																										
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																										
<table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">Louvenia Carter-Dawson</td> <td style="width: 15%;">Ph.D.</td> <td style="width: 25%;">Staff Fellow</td> <td style="width: 10%;">LVR</td> <td style="width: 10%;">NEI</td> </tr> <tr> <td>Other:</td> <td>Toichiro Kuwabara</td> <td>M.D.</td> <td>Head, Section on Experimental Pathology</td> <td>LVR</td> <td>NEI</td> </tr> <tr> <td></td> <td>Paul J. O'Brien</td> <td>Ph.D.</td> <td>Research Chemist</td> <td>LVR</td> <td>NEI</td> </tr> <tr> <td></td> <td>John G. Bieri</td> <td>Ph.D.</td> <td>Chief, Section on Nutritional Biochemistry</td> <td>LNE</td> <td>NIAMDD</td> </tr> </table>			PI:	Louvenia Carter-Dawson	Ph.D.	Staff Fellow	LVR	NEI	Other:	Toichiro Kuwabara	M.D.	Head, Section on Experimental Pathology	LVR	NEI		Paul J. O'Brien	Ph.D.	Research Chemist	LVR	NEI		John G. Bieri	Ph.D.	Chief, Section on Nutritional Biochemistry	LNE	NIAMDD
PI:	Louvenia Carter-Dawson	Ph.D.	Staff Fellow	LVR	NEI																					
Other:	Toichiro Kuwabara	M.D.	Head, Section on Experimental Pathology	LVR	NEI																					
	Paul J. O'Brien	Ph.D.	Research Chemist	LVR	NEI																					
	John G. Bieri	Ph.D.	Chief, Section on Nutritional Biochemistry	LNE	NIAMDD																					
COOPERATING UNITS (if any)  Laboratory of Nutrition and Endocrinology, NIAMDD																										
LAB/BRANCH Laboratory of Vision Research																										
SECTION Section on Experimental Pathology																										
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20014																										
TOTAL MANYEARS: 1.3	PROFESSIONAL: 1.1	OTHER: 0.2																								
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																										
SUMMARY OF WORK (200 words or less - underline keywords)  <p>The effects of <u>vitamin A deficiency</u> on <u>rhodopsin</u>, <u>opsin</u>, and maintenance of <u>retinal structure</u> were examined in rats reared in low levels of cyclic light. The results show a rapid decline in the level of rhodopsin followed by a slower rate of opsin loss. Disruption of retinal structure more closely paralleled the loss of opsin. Both <u>rod</u> and <u>cone</u> photoreceptors are affected by vitamin A deficiency but rods are affected to a greater extent.</p> <p>Corneas of vitamin A deficient rats (low levels of retinoic acid) show several abnormalities before xerophthalmia is evident. The peripheral stroma becomes <u>vascularized</u>, and the <u>keratocytes</u> and <u>histocytes</u> show electron dense inclusion bodies. Corneal epithelial cells show an accumulation of electron lucent vacuoles and an increase in the extracellular space. The surface of the epithelium is highly irregular with many partially detached cells.</p>																										

Project Description:

Objectives: A large number of people, especially children, suffer from vitamin A deficiency in several less developed areas of the world, such as Asia and Central America. This deficiency results in poor or complete loss of vision, xerophthalmia, and keratomalacia. This project was designed to investigate the effects of vitamin A deficiency on rhodopsin and opsin levels, and maintenance of retinal and corneal structure at various stages of deficiency.

Methods Employed: For retinal studies, pregnant rats were placed on a vitamin A free diet (basal diet) seven days before delivery. The male offspring were weaned at three weeks of age and placed in one of three groups: 1) basal diet, 2) basal diet plus retinoic acid at 35 days of age (4mg/kg diet) and 3) basal diet plus retinyl palmitate at weaning (21 days, 4mg/kg diet). The rats were fed the diet and water ad libitum. They were maintained in cyclic light--12 hours light, 12 hours dark--at a cage illumination of 1.5-2 foot-candles. The retinas were examined by light and electron microscopy, and levels of rhodopsin and opsin were measured.

For studies of vitamin A deficient corneas, rats were reared as described above. Male and female rats were weaned and placed in one of three groups. However, rats receiving only the basal diet were supplemented with retinoic acid (4mg/kg diet) when a loss in weight was detected. Some rats remained on this diet for 1 to 18 weeks. Between 1 and 3 weeks some rats (largely males) were placed on 400 µg, 200 µg, or 50 µg/kg diet mix of retinoic acid. Others (largely females) were given 800 µg/kg diet mix at 18 weeks. The cornea were examined by light, electron, and scanning microscopy at various ages on the diet.

Major Findings: The levels of rhodopsin and opsin and retinal structure are affected in vitamin A deficient rats reared in nondamaging levels of cyclic illumination (1.5-2 foot-candles). This study shows several findings not seen in previous studies. Rhodopsin levels decrease in deficient retinas but 20% of normal levels is maintained through 39 weeks on the deficient diet. Opsin levels decrease at a slower rate but reach 20% of control levels by 32 weeks. Despite the decrease of rhodopsin levels, obvious deterioration of disc structure is not observed until 16 weeks of deficiency. The disruption of structure is predominately localized in discs of the distal third. Degeneration of rod and cone nuclei is also seen, however, rod nuclei degenerate at a faster rate than cone nuclei in the central and peripheral retina.

The corneas of vitamin A deficient rats are also affected. Microscopic examination of the deficient corneas, before xerophthalmia is apparent, shows vascularization of the peripheral cornea and accumulation of electron dense inclusion bodies in the keratocytes and histocytes. Corneal epithelial cells show numerous small electron lucent vacuoles within the cytoplasm and an increase in the extracellular space. Scanning microscopy reveals an abnormally large number of epithelial cells elevated and partially detached from the underlying cells.

Significance to Biomedical Research and the Program of the Institute:

Results from these studies will provide further insight into the role of vitamin A in the maintenance of the neural retina, pigment epithelium and cornea. These studies will also provide information on the early histological, cytological, and surface changes which occur before poor vision, xerophthalmia, and keratomalacia are manifested,

Proposed Course: It is generally believed that vitamin A deficiency can result in xerophthalmia and keratomalacia. It is not clear whether these are primary or secondary changes due to infection. This question will be further examined in vitamin A deficient rats reared in a germ free environment. In addition, the conjunctiva will be examined at various stages of deficiency in rats reared in a germ free environment and standard laboratory conditions.

NEI Research Program: Retinal and Choroidal Diseases--Visual Cells and Pigment Epithelium

Publications:

Carter-Dawson L, Kuwabara T, O'Brien PJ, Bieri JG: Structural and biochemical changes in vitamin A deficient rat retinas. Invest Ophthalmol Visual Sci (in press).

Carter-Dawson L, Kuwabara T, Bieri JG: Early histological and surface changes in vitamin A deficient rat corneas. Invest Ophthalmol Visual Sci (in press).

Carter-Dawson L, LaVail MM, Sidman RL: Differential effect of the rd mutation on rods and cones in the mouse retina. Invest Ophthalmol Visual Sci 17:489-498, 1978.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00129-06 LVR
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PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Anatomical and Pathological Studies of Ocular Tissues

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Toichiro Kuwabara	M.D.	Head, Section on Experimental Pathology	LVR	NEI
Other:	W. Gerald Robison, Jr.	Ph.D.	Geneticist/Cell Biologist	LVR	NEI
	Louvenia Carter-Dawson	Ph.D.	Staff Fellow	LVR	NEI
	Minoru Tanaka	M.D.	Visiting Scientist	LVR	NEI
	Yoshitaka Ohnishi	M.D.	Visiting Scientist	LVR	NEI
	Teruo Tanishima	M.D.	Visiting Scientist	LVR	NEI
	Fujiko L. Huang	M.D.	Visiting Scientist	LVR	NEI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Vision Research

SECTION

Section on Experimental Pathology

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

5.5

PROFESSIONAL:

4.5

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS

☒ (b) HUMAN TISSUES

☐ (c) NEITHER

☐ (a1) MINORS

☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Histopathological studies were conducted on numerous pathological human eyes by transmission and scanning electron microscopy, histochemistry and histological sectioning. Studies were completed on corneal dystrophy, amyloidosis of the orbit, cryptococcosis, Niemann-Pick Disease, eyes with senile changes, cataractous lenses, retinitis pigmentosa and eyes with papilledema. In addition other studies were on lipogenesis of the corneal stroma cell, lamellar inclusion bodies induced by AY9944, ATPase in cultured lens cells (lentoid body), axoplasmic flow in experimental glaucoma and phagocytosis of the ciliary epithelium.

Project Description:

Objectives: Clarification of normal structure and function of each cell of the eye is fundamental to understanding the pathophysiology in various eye diseases. Also, a systematic study of the eye with naturally occurring diseases may directly lead to the elucidation of the pathogenesis involved.

Methods Employed: A large number of clinicopathological specimens sent to this laboratory from various eye research centers throughout the world were studied. Details on individual experiments on animals are described under the headings of Major Findings.

These eye tissues were fixed in glutaraldehyde solution and processed for transmission and scanning electron microscopy. Depending on specific diseases various types of histochemical reactions were applied on cryo-, frozen, paraffin and plastic sections.

Major Findings:

I. Studies on the normal eye

A chapter entitled "The Eye" in a textbook of histology was published. This chapter mainly describes findings in the human eye and contains comprehensive new electron micrographs with updated interpretations.

Age-related cytological and histological changes of the eye were summarized for the Symposium on Biology of Special Senses in Aging which was held at the University of Michigan. A paper including a systematic description of the age related changes of the eye is believed to be the first.

Fine structural details of the developing eye, especially of the anterior portion, were extensively studied. This study revealed that the connective tissue of the trabecular meshwork is formed mainly by the endothelium-like cells at the angle. The angle and the anterior surface of this iris are covered with thin, stellate cells. Also, it was demonstrated that no cleavage of the mesenchymal tissue occurs during the development of the angle. The result was presented at the Third International Congress of Eye Research in Osaka, Japan.

II. Studies on the cornea

Cases of several endothelial dystrophy including a case of amorphous posterior endothelial dystrophy were extensively studied.

Fine structural findings in the human cornea were summarized. This paper emphasized the dynamic moving action of the stroma cell and relatively mild phagocytic activity in this cell. Also, a cytological explanation of the transparency of corneal tissue was presented.

An early wound healing mechanism of small wounds which had been made on the posterior surface of the rabbit cornea was studied. The cut edges of Descemet's membrane curled toward the anterior chamber and a tissue gap measuring about 200  $\mu\text{m}$  was formed immediately following the wounding. The endothelial cells in the vicinity of the wound began to slide along Descemet's membrane and reached the cut edge three hours after the wounding. The sliding of the endothelial cells continued until the tissue defect was filled by the twelfth hour. By then the cells facing the anterior chamber became the covering endothelium by forming conspicuous apicolateral junctions and basal lamina. Whereas, the cells piled in the tissue defect began to show a fibroblast-like appearance--losing junctions. These cells produced abundant basal lamina-collagen substances among them. The sliding and transformation of the endothelium occurred without mitotic activity.

Aberrant lipogenesis, a protective reaction of cells by synthesis of triglycerides utilizing abnormally present fatty acids, was reported by Cogan and Kuwabara in 1958 (Science 120:321). In order to elucidate cytological details of this phenomenon, small amounts of sodium oleate were injected into the corneal stroma of the rabbit, and the fat-forming cells were examined by electron microscopy. When tritiated oleate was used, the cytoplasm of stroma cells in the vicinity of injection became diffusely radioactive immediately following the experiment. The radioactivity was gradually concentrated in fat droplets. The cytoplasm of the corneal stroma which contains relatively sparse microorganelles, began to form a small clear space measuring about 0.05  $\mu\text{m}$  in diameter in the matrix without correlation to any microorganelle. These spaces never became radioactive. The space may be the site of glyceride formation. These spaces became membrane-bound oil droplets, measuring average 0.5 - 1.0  $\mu\text{m}$  in diameter, within 5-6 hours. The number and size of oil droplets increased for a few days and stayed intracellularly for a long period of time without causing any degenerative effect to the cell. In earlier studies the fatty substance had been proven to be mainly triglyceride (Arch Ophthalmol 61:361, 1958). Some long-standing fat droplets developed a few myelin figures. These lamellar membranes were heavily incorporated with the radioactive oleate.

### III. Experimental Niemann-Pick disease

Intraperitoneal injection of AY 9944, an inhibitor of cholesterol biosynthesis, induced abundant lamellar inclusion bodies in various cells of the albino rat. Morphological and biochemical studies revealed that the pathogenesis of the treated animal resembled that of Niemann-Pick disease which is characterized by a deficiency in cholesterol and sphingomyelin metabolism. The inclusion bodies developed first in the smooth endoplasmic reticulum. Abundant inclusion bodies were equally produced in the nonneural cells which have totally different cytoplasmic structures from those of the neural cells. Cytologic appearances of the pigment epithelial cell and the lens fiber were specifically compared.

#### IV. Studies on the lens

Numerous lenses with senile cataracts have been submitted to this laboratory for cytological studies from members of the Cooperative Cataract Research Group. Lenses were studied grossly, photographed, and examined by histology and electron microscopy. Flat preparations and histological sections revealed that the epithelial cells became progressively sparse with age. Several acellular foci were formed. Electron microscopy of the remaining cells showed that the cytoplasm had lost the normal structure. The cataractous changes of the anterior cortex were regularly localized beneath the abnormal epithelium. These findings indicated that cytological changes in the epithelium preceded the occurrence of the senile changes in the lens fibers.

Lentoid bodies, lens-like aggregation of the cultured lens epithelial cells of normal and Nakano cataractous strain mice were studied by transmission and scanning electron microscopy. Cells of the lentoid body of the mouse lens epithelium tend to form random aggregations, and marked degeneration in the central zone was present. A considerable difference in the structure of lentoid bodies was noted between those of the mouse and of the chick.

Although the appearances of cells constituting lentoid bodies of both normal and cataractous mice were similar, electron microscopic histochemistry revealed that there is a marked difference in the ouabain-sensitive ATPase activity. The lead particles produced by the action of ATPase were abundantly present in the vicinity of cell membranes of lentoid body cells of the normal mouse, whereas they were absent in cells of Nakano strain mouse. This observation is consistent with the finding that an ATPase inhibitor which is present in the lens of Nakano strain was carried into the cultured cells.

#### V. Glaucoma study

Numerous clinicopathological materials of papilledema were studied electron microscopically. The increased mass of the disc consists of axonal hydrops, aggregation of mitochondria and dense bodies, and disorganization of axoplasm. Although the literature contains abundant speculations on the pathogenesis of papilledema, recent experimental observations point to an underlying interruption of axonal flow as the most plausible hypothesis for most types of papilledema.

The anterior optic nerve and the macular region of the retina of glaucomatous eyes of rhesus monkeys have been examined by light and electron microscopy. The eyes were examined three to eleven weeks after the onset of sustained elevation of intraocular pressure and longer duration of glaucoma. Eyes with a lesser elevation of intraocular pressure and shorter duration of glaucoma showed changes sharply localized to the axon bundles in the scleral lamina cribrosa. Accumulation of mitochondria and of dense bodies occurred anterior and posterior to collagenous septae. The location of these changes is in agreement with the localization of block of axoplasmic transport identified by autoradiographic studies. It is speculated that these cytological changes reflect blockage of axoplasmic flow in the optic nerve of eyes with glaucoma.

A marked hemosiderin in the nonpigmented ciliary epithelium (NPCE) is one of the regular histopathologic findings in hemorrhagic eyes. It is also common to find similar particles in NPCE cells of normal elderly humans. The deposited substances are strongly electron-dense and grouped with lysosomes and lipofuscin. In addition, X-ray microanalysis reveals that the substance contains iron. A hemoglobin solution and particles in various sources were injected into the posterior chamber of rhesus monkeys in order to study the phagocytic activity of the NPCE cell. The injected substance seemed to enter the cell through the lateral cell membranes of the NPCE about 24 hours after injection and then were rapidly surrounded by the plasma membrane. These membrane-bound particles were eventually fused with lysosomes, which are numerous and contain various lysosomal enzymes in the normal condition. The phagocytized substance seemed to remain within the cell for a long period of time. This study has revealed that the NPCE cells have a slow and steady phagocytic activity. It is suggested that this function is to remove and detoxicate foreign substances, which may arise as a result of minute degeneration or hemorrhage within the ocular cavity.

#### VI. Retinitis pigmentosa

In collaboration with the staff of the Section of Retinal and Corneal Metabolism of LVR, eyes of a retinitis pigmentosa patient were studied. Details of these experiments are presented elsewhere by Dr. Chader, Project No. Z01 EY-00148-05 LVR.

#### VII. Amyloidosis

An unusual case of amyloidosis was studied. Electron microscopic examination of the conjunctival tissue resulted in the identification of a cell in intimate contact with the aggregations of amyloid fibrils, containing intracellular masses of amyloid fibrils, and having tufts of amyloid fibrils packed into its cytoplasmic invaginations. These morphologic characteristics suggested the possibility of localized synthesis of amyloid fibrils by these cells.

Significance to Biomedical Research and the Program of the Institute: The staff of this section is able to pursue a multidisciplinary study to attack problems which are directly related to clinical ophthalmology. Further clarification of the normal and abnormal structure and function of ocular tissues and cells is a significant part of eye research.

Proposed Course: Similar projects are actively ongoing and will be continued in the next fiscal year.

NEI Research Program: Retinal and Choroidal Diseases--Developmental and Hereditary Disorders/Inflammatory Disorders/Uveal Tract; Corneal Diseases--Corneal Edema, Dystrophies, and Inherited Disorders/Corneal Transplantation and Stromal Injury and Repair/Tumors and Other Lid, Conjunctival, and Orbital Problems; Cataract--The Normal Lens/Cataract Induced by Drugs and Radiation and Occurring Secondary to Other Eye Disorders; Glaucoma--Etiology of Glaucoma (Primary Open-Angle Glaucoma/Secondary Glaucomas)

Publications:

Kuwabara T, Cogan DG: The eye, in Weiss L, Greep RO (eds): Histology 4th ed, New York, McGraw Hill, 1977, pp 1119-1164.

Kuwabara T: Age-related changes of the eye. Proceedings of Symposium On Biology of Special Senses in Aging, Ann Arbor, University of Michigan Press, (in press).

Kuwabara T: Current concepts in anatomy and histology of the cornea. Contact Lens and Intraocular Lens Medical J 14:1-32, 1978.

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Huang FL, Russell P, Kuwabara T: Fine structure of lentoid bodies derived from normal and cataractous mouse lenses. Exp Eye Res (in press).

Huang FL, Chylack LT, Kuwabara T: Pathological changes of the epithelium with age and in senile cataract. Am J Ophthalmol (in press).

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Gaasterland D, Tanishima T, Kuwabara T: Axoplasmic flow during chronic experimental glaucoma. 1. Light and electron microscopic studies of the monkey optic nervehead during development of glaucomatous cupping. Invest Ophthalmol Visual Sci (in press).

Bergsma DR, Wiggert BN, Funahashi M, Kuwabara T, Chader GJ: Vitamin A receptors in normal and dystrophic human retina. Nature 265:66-67, 1977.

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Kaiser-Kupfer M, McAdam KPWJ, Kuwabara T: Localized amyloidosis of the orbit and upper respiratory tract. Am J Ophthalmol 84:721-728, 1977.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00149-05 LVR												
PERIOD COVERED October 1, 1977 to September 30, 1978														
TITLE OF PROJECT (80 characters or less)  Ultrastructure and Function of the Pigment Cells of the Eye														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">W. Gerald Robison, Jr.</td> <td style="width: 35%;">Ph.D. Geneticist/Cell Biologist</td> <td style="width: 15%;">LVR NEI</td> </tr> <tr> <td>Other:</td> <td>Toichiro Kuwabara</td> <td>M.D. Head, Section on Experimental Pathology</td> <td>LVR NEI</td> </tr> <tr> <td></td> <td>John G. Bieri</td> <td>Ph.D. Chief, Section on Nutritional Biochemistry</td> <td>LNE NIAMDD</td> </tr> </table>			PI:	W. Gerald Robison, Jr.	Ph.D. Geneticist/Cell Biologist	LVR NEI	Other:	Toichiro Kuwabara	M.D. Head, Section on Experimental Pathology	LVR NEI		John G. Bieri	Ph.D. Chief, Section on Nutritional Biochemistry	LNE NIAMDD
PI:	W. Gerald Robison, Jr.	Ph.D. Geneticist/Cell Biologist	LVR NEI											
Other:	Toichiro Kuwabara	M.D. Head, Section on Experimental Pathology	LVR NEI											
	John G. Bieri	Ph.D. Chief, Section on Nutritional Biochemistry	LNE NIAMDD											
COOPERATING UNITS (if any)  Laboratory of Nutrition and Endocrinology, NIAMDD														
LAB/BRANCH Laboratory of Vision Research														
SECTION Section on Experimental Pathology														
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20014														
TOTAL MANYEARS: 2.2	PROFESSIONAL: 1.2	OTHER: 1.0												
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS														
SUMMARY OF WORK (200 words or less - underline keywords)  <p>             The possible roles of <u>vitamin E</u> (<math>\alpha</math>-tocopherol) in protecting <u>photoreceptor membranes</u> from autoxidation, in influencing pigment epithelial storage of <u>vitamin A</u>, and in retarding the accumulation of <u>lipofuscin</u> (<u>aging pigment</u>) in the <u>retina</u> were investigated. Rats were deprived of vitamin E while receiving marginal or fully adequate amounts of vitamin A for 3, 5, and 8 months. By 5 months the retinas of vitamin E-deficient rats exhibited altered <u>outer segment membranes</u>, loss of photoreceptor nuclei, and the accumulation in the <u>pigment epithelium</u> of unusually large numbers of intracellular granules showing lipofuscin-specific <u>autofluorescence</u>. Extraordinary accumulations of aging pigment occurred also in <u>extraocular muscle</u>, <u>uterus</u>, <u>heart</u>, <u>liver</u> and <u>brain</u>. Vitamin A-specific autofluorescence of <u>retinal pigment epithelium</u> and <u>liver</u> were higher in vitamin E-fed compared to vitamin E-deprived rats. Thus, vitamin E probably inhibited the peroxidative loss of vitamin A in both these tissues. In addition, vitamin E protected the photoreceptor cells from membrane disintegration and retarded aging pigment formation in the retinal pigment epithelium.           </p>														

Project Description:

Objectives: Study the structural and functional interrelationships that exist between the pigment epithelium and the visual cells of the eye. We propose to examine how the pigment epithelial cells are involved in the maintenance of photoreceptor cells and what specific functions are lacking in various experimental and pathological cases.

Methods Employed: Rats were made vitamin E deficient, and their retinas as well as other tissues were observed for changes from control tissues which might shed light on the roles of vitamin E and of the retinal pigment epithelium in E sufficient animals. In order to counteract the known effects of low vitamin E on vitamin A storage, half of the rats were fed enough vitamin A to maintain normal storage levels, and half were given only marginal levels. The tissues were examined in frozen section for the autofluorescence specific to lipofuscin, were examined by light microscopy to measure and count retinal components including cytoplasmic inclusions and photoreceptor nuclei, and were examined by electron microscopy for ultrastructural changes.

Major Findings: The retinas of vitamin E-deficient rats exhibited significant losses of photoreceptor nuclei from the outer nuclear layer, ultrastructural alterations in the membranes of photoreceptor outer segments, and unusually large accumulations of lipofuscin (aging pigment) granules as well as loss of stored vitamin A in the retinal pigment epithelium.

These results suggest accelerated autooxidation of photoreceptor membranes and an increased accumulation of their peroxidized products along with the peroxidized products of stored vitamin A in the pigment epithelium in the form of lipofuscin granules. Thus, it appears that normally vitamin E protects the retinal membranes from autooxidation and retards the accumulation of aging pigment and loss of stored vitamin A in the pigment epithelium.

Significance to Biomedical Research and the Program of the Institute:

It is becoming increasingly evident that the pigment epithelium plays important and dynamic roles in the maintenance and function of photoreceptor cells. The fact that vitamin E deficiency affected only the photoreceptor and the pigment epithelial cells indicates a close interrelationship of these retinal components, especially in lipid metabolism and membrane dynamics. Not only were vitamin A storage levels lowered in the pigment epithelium, but the photoreceptor cells lost structural integrity. Not only were outer segment membranes disrupted, but the pigment epithelium which ingests and degrades such membranes became filled with lipofuscin which represents the end stage of membrane peroxidation. It is clear that one cannot understand fully the metabolic or structural state of photoreceptor cells without understanding the state of pigment epithelial cells. Surely the damaging effects of various light regimes on the retina cannot be understood without a knowledge of vitamin E and vitamin A levels in the plasma and in the retinal pigment epithelium.

Proposed Course: Rats will be deprived of vitamin E and maintained under various light cycles and intensities to see how light damage to the retina is altered without the protective influence of  $\alpha$ -tocopherol. Vitamin A levels will be varied and carefully monitored.

A preliminary report suggests that lipofuscin granules contain retinoyl complexes deriving from retinoic acid. Rats will be fed retinoic acid in lieu of vitamin A for 3, 5, and 8 months and the amount and characteristics of their lipofuscin accumulated in retinal pigment epithelial cells will be examined.

NEI Research Program: Retinal and Choroidal Diseases--Visual Cells and Pigment Epithelium

Publications:

Robison WG Jr, Kuwabara T: Vitamin A storage and peroxisomes in retinal pigment epithelium and liver. Invest Ophthalmol Visual Sci 16:1110-1117, 1977.

Robison WG Jr, Kuwabara T: A new, albino-beige mouse: Giant granules in retinal pigment epithelium. Invest Ophthalmol Visual Sci 17:365-370, 1978.

Shinohara T, Robison WG Jr, Piatigorsky J:  $\alpha$ -Crystallin synthesis and vacuole formation during induced opacification of cultured embryonic chick lenses. Invest Ophthalmol Visual Sci 17:515-522, 1978.

Robison WG Jr, Kuwabara T, Bieri JG: Vitamin E deficiency and the retina: Photoreceptor and pigment epithelial changes. Invest Ophthalmol Visual Sci (in press).



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00044-02 LVR
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PERIOD COVERED  
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)  
Study of the Rod Outer Segment Renewal System of the Normal and Dystrophic Rat

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Makoto Tamai M.D. Visiting Scientist LVR NEI

Other: None

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Vision Research

SECTION

Section on Experimental Pathology

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS

☐ (b) HUMAN TISSUES

☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The role of the pineal body, which is considered to be a control center of the circadian rhythm, the rod outer segment (ROS) renewal system was studied with pinealectomized and superior cervical ganglionectomized rats. The renewal system of ROS was also examined from the developmental aspect in control and dystrophic rats. In vitro regeneration of microvilli of the pigment epithelium (PE) was observed by using the organ culture technique in these animals.

Project Description:

Objectives: The length of the rod outer segment is kept constantly balanced by two processes: (1) disc renewal from the inner segment of the photoreceptor cell, and (2) shedding and phagocytosis by the pigment epithelium. The renewal process was reported to be controlled by a circadian rhythm, but remaining to be answered were the questions of when this process is established after birth and what organs participated in the process. Also, it was not known whether this renewal process exists in the dystrophic RCS rat or is developed before the photoreceptor degenerates. These problems were examined in the Sprague-Dawley rat and the control (rdy<sup>+</sup>) and dystrophic RCS rat by light and electron microscopy.

Methods Employed: The pineal gland is thought to be the hormonal center of the circadian rhythm. Thus, the daily rhythm of the ROS shedding and phagocytosis was examined and compared in pinealectomized and sham-operated SD rats. In addition, the number of phagosomes in the PE was counted in each stage of rod outer segment development up to 50 days after birth in both control and dystrophic rats. The amount of phagosomes was determined by light microscopy.

For observing the regeneration of microvilli of the PE, the neural retina and pigment epithelium were separated, recombined, and cultured in vitro. The surface of the PE was observed by scanning and transmission electron microscopy at varying periods of incubation from 12 hours to 5 days.

Major Findings: Diurnal patterns of retinal outer segment shedding and phagocytosis by the pigment epithelium were quantitatively similar in pinealectomized, superior cervical ganglionectomized and sham-operated rats. Sharp increases in the number of large phagosomes were observed soon after the lights were turned on in the three sets of animals. Pinealectomized animals kept in constant darkness over a 24-hour period also exhibited normal shedding patterns. The results indicate the pineal gland does not appear to influence the renewal process.

During the postnatal development of ROS, the number of phagosomes found increased steadily in the control rats until adult levels were reached at 35 days. In the dystrophic animals the maximum number was observed 15 days and showed no further increases with age. The biorhythms were completed around 35 days after birth in the control rat but were not completed in the dystrophic animals because of the photoreceptor cell degeneration.

Organ culture of control retina-PE explants caused a burst of ROS shedding and phagocytosis, with the maximum number of phagosomes appearing at two hours of incubation. The dystrophic retina-PE explants exhibited a very small peak at four hours. These results confirmed the previous observations in recombination organ cultures of dystrophic retinas with control PE and control retina with dystrophic PE that the retina of the dystrophic rat was apparently normal while the PE carried the defect.

The microvilli of the pigment epithelium were easily torn off by mechanical insult but regenerated rapidly in vitro. By 12 hours of incubation, the regeneration of villous processes was evident, and by 24 hours the surface was densely covered with these processes. In the dystrophic pigment epithelium, the period required for regeneration of microvilli was practically the same as the pigment epithelium from control animals.

Significance to Biomedical Research and the Program of the Institute: The study of the photoreceptor renewal process in normal and dystrophic animals may lead us to an understanding of the human retinal dystrophies.

Proposed Course: This research will be continued at the Department of Ophthalmology, Tohoku University School of Medicine, upon the investigator's return.

NEI Research Program: Retinal and Choroidal Diseases--Visual Cells and Pigment Epithelium

Publications:

Tamai M, Takahashi J, Noji T, Mizuno K: Development of photoreceptor cells in vitro. Influence and phagocytic activity of homo- and heterogenic pigment epithelium. Exp Eye Res 26:581-590, 1978.

Tamai M, Teirstein P, Goldman A, O'Brien P, Chader G: The pineal gland does not control rod outer segment shedding and phagocytosis in the rat retina and pigment epithelium. Invest Ophthalmol Visual Sci 17:558-562, 1978.

Tamai M: Regeneration of microvilli of the pigment epithelium in vitro. Proc XXIII Int Cong Ophthalmol, (in press).



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRANURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00065-01 LVR
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less)  Physiological and Anatomical Studies of the Visual System of Primates		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  PI: Francisco M. de Monasterio M.D., D.Sc. Visiting Scientist LVR NEI  Other: None		
COOPERATING UNITS (if any)  Stanley J. Schein, M.D., Ph.D., Department of Psychology, MIT, Cambridge, Massachusetts		
LAB/BRANCH Laboratory of Vision Research		
SECTION Section on Neurophysiology		
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20014		
TOTAL MANYEARS: 1.1	PROFESSIONAL: 1.1	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)  This project aims to study the anatomical and physiological organization of neurons of the visual system of non-human primates that can serve as a model for the human visual system. The project gives emphasis to the <u>chromatic</u> and <u>spatial properties</u> and <u>central projections</u> of neurones of the <u>retina</u> , <u>lateral</u> <u>geniculate body</u> , <u>striate cortex</u> and <u>extra-striate cortex</u> .		

Project Description:

Objectives: To study the neural organization underlying visual perception in primates.

Methods Employed: Intracellular and extracellular recordings from single neurons, intracellular staining with fluorescent dyes, extracellular recordings of mass responses; correlation of the distribution of single cell varieties and morphological cell types as seen by electron and light microscopy; autoradiographic observations of the distribution of radionuclide-labelled neurons.

Major Findings:

## I. Spatial properties of ganglion cells of the macaque retina:

Macaque ganglion cells having a concentrically-organized, center-surround receptive field can be classed as X- or Y-cells on the basis of the linearity or non-linearity of their spatial summation to a null test of alternating contrast and drifting sinusoidal gratings. When an alternating-phase bipartite-field positioned at the middle of the receptive field was used as a stimulus, X-cells had a null position (no response) while Y-cells showed a doubling of the response frequency (second harmonic). When drifting gratings of low contrast were used as a stimulus, X-cells showed a periodic modulation of their discharge, having the same value for different spatial frequencies, whereas Y-cells showed a large increase in the mean value of their discharges. X-cells had opponent-color responses which received cone-specific signals, i.e. center and surround responses were mediated by input from spectrally different types of cone, while Y-cells had broad-band spectral responses receiving mixed-cone input, i.e. center and surround responses were totally or partly mediated by signals from the same type(s) of cone. In most Y-cells, the spatially-opponent responses from the center and the surround were mediated by the same types of cone and were thus spectrally non-opponent; other Y-cells showed spectral opponency, as one of the types of cone mediating responses of one region of the receptive field (e.g. center) was absent in the responses of the other region (e.g. surround). Pure-center and pure-surround responses of Y-cells have a fast decay and show conspicuous transients at stimulus offset and onset; their response properties are consistent with the presence of excitatory and inhibitory processes within the network of each one of the opponent regions of the receptive field. Pure responses of X-cells have a slow decay and show fewer transients, especially at stimulus offset, and their properties are consistent with the presence of either excitatory or inhibitory processes within each one of the opponent regions of the receptive field. Sensitivity profiles of the spatial distribution of pure-center and pure-surround responses elicited in conditions of chromatic adaptation of the opponent responses show that opponent-color Y-cells have a unimodal center and a unimodal surround profile which resemble Gaussian distributions of different amplitude and extent, whereas opponent-color X-cells have a unimodal center and a bimodal surround profile. The presence of a bimodal profile in the latter cells gives them a higher spatial resolution than that in the presence of a unimodal surround profile.

The spatial properties of X-cells indicate that these neurons have a better resolution of equal-luminance chromatic gratings of low spatial frequency and of equal-chromaticity luminance gratings of high spatial frequency. These properties are consistent with those of the human visual system when examined with luminance and chromatic gratings projected in the foveal region. Responses receiving contribution from both opponent regions of the receptive field (mixed responses) have a different time course and pattern in X- and Y-cells. Mixed responses of Y-cells show a discontinuity in cell firing during the transient on-component of cell activity which has a higher sensitivity than other waveform changes produced by the concurrent stimulation of the opponent mechanisms. The discontinuity occurs with stimulus conditions that also elicit proximal negative responses in the local electroretinogram and it appears to be due to a centrally-located process having some degree of rectification.

About 10% of macaque ganglion cells appear to lack a typical center-surround organization. These cells, as a single population, had a diffuse extra-foveal distribution and were less frequently observed in the foveal region. Three groups of such cells were distinguished. One group had spectrally-opponent responses mediated by mechanisms having similar or identical spatial distributions and response latencies. These cells do not respond to white light. They predominate in the central retina and usually receive input from all three types of retinal cones; they have a linear summation of incoming signals over the receptive field; they lack inputs from retinal rods. A second group showed on-off responses to small and large stimuli. One subgroup had excitatory or inhibitory on-off responses and a silent inhibitory surround which tended to suppress cell responses and maintained activity. They are observed throughout the central retina, including the fovea; they receive input from green- and red-sensitive cones but not from blue-sensitive cones; they have a non-linear summation over the receptive field. Another subgroup of these neurons lacked spontaneous activity and any type of surround. They are encountered at a retinal depth more sclerad than that of other spike-generating neurons and could not be antidromically stimulated from the optic tract or more central structures, suggesting absence of an optic-nerve projecting axon. These cells lack input from blue-sensitive cones and have a non-linear spatial summation. The third group of cells lacking a center-surround organization were predominantly inhibited by moving stimuli and failed to respond to stationary flashing stimuli. They appear to be more common towards the peripheral retina.

## II. Central projections of the various types of macaque ganglion cells:

The central projections of macaque ganglion cells described in previous studies were examined by the electrical stimulation of the optic tracts, lateral geniculate body, and superior colliculus. Of the six main types of ganglion cells described to date in the macaque retina, the results show that most cells project to the lateral geniculate body (LGB), whereas a fraction of the cells appear to project to both the LGB and the superior colliculus (SC). The projection and main characteristics of these ganglion cells are listed below:

Type I cells (opponent-color, cone-specific, linear summation, center-surround) project to the LGB but not the SC with slow conduction organization velocities. Type II cells (opponent-color, cone-specific, linear summation, no center-surround) project to the LGB but not the SC with medium conduction velocities. Type III cells (non-opponent-color, cone-mixed, non-linear summation, center-surround organization) project to the LGB and a fraction also to the SC with fast conduction velocities. Type IV cells (opponent-color, cone-mixed, non-linear summation, center-surround organization) project to the LGB but not the SC with fast conduction velocities. Type V (non-opponent-color, cone-mixed, non-linear summation, no center-surround organization, on-off responses) project to both the LGB and SC with slow conduction velocities. Type VI cells (non-opponent-color, motion-sensitive responses, no center-surround organization) apparently project to the LGB but not the SC with very fast conduction velocities.

### III. Intracellular recordings of retinal neurons of the perfused eyecup:

This project has been directed to the characterization and identification of intracellularly recorded cells of the macaque monkey retina using the isolated and arterially-perfused eyecup preparation. Several types of S-potentials have been obtained in this system; those showing absence of a center-surround organization have been tentatively classed as horizontal cell responses pending dye-injection identification. These S-potentials show input from both rods and cones, or apparently from cones alone. Other S-potentials show opponent-color responses (biphasic) mediated by signals from different cones indicating the presence of spectral interactions at the distal levels of the macaque retina. A few other S-potentials show a center-surround organization; they have been tentatively identified as bipolar cells pending dye-injection identification. Intracellular recordings from ganglion cells have also been obtained. Dye-injection of "blue-ON-center, yellow-OFF-surround" ganglion cells having color-opponent responses indicate that these correspond to diffuse stratified ("parasol") ganglion cells.

### IV. ON/OFF asymmetry of the blue-cone pathway:

Activity mediated by signals from blue cones tends to show little or no OFF responses at both the proximal and distal levels of the retina. The majority of ganglion cells receiving input from blue cones have opponent-color responses; whereas 91% of "blue-center" cells are ON-center and 9% are OFF-center, "green-" and "red-center" cells appear to be ON- or OFF-center with rather similar incidence (58%/42% and 61%/39%). In addition, local mass responses obtained at more distal levels show that the local electroretinogram mediated by blue-cone signals lacks a d-wave (being similar to that mediated by rods), whereas this OFF-response (typical of the cone-type ERG of light-adapted retinas) is present in mass potentials mediated by signals from green and red cones. "Blue-ON-center" cells have larger cell bodies and faster conduction velocities than other opponent-color cells, and dye-injections of Procion Yellow show that they correspond to diffuse stratified ganglion cells whose dendrites stratify in the scleral half of the inner plexiform layer. "Red-" or "green-ON-center" ganglion cells, in contrast, have smaller cell

bodies and slower conduction latencies; preliminary stainings with Procion Yellow suggest that these may correspond to midget ganglion cells whose dendrites stratify within the vitread half of the inner plexiform layer. The latter stratification resembles that of similar cells of the cat retina, in which OFF-center cells stratify in the sclerad half and ON-center cells in the vitread half of the inner plexiform layer. Thus, ganglion cells whose ON-centers receive input from blue cones show a reversal of the ON/OFF lamination of this layer, resembling a similar reversal found in the parvocellular layers of the lateral geniculate body of this species. These asymmetries, if related, could indicate that most blue-cone signals to ganglion cells are transmitted via a diffuse flat cone bipolar having a cone-specific direct input from blue cones, although if such bipolars are indeed of the hyperpolarizing type (as recent work has suggested) their contacts with "blue-ON-center" ganglion cells should be sign-inverting to account for the asymmetry of response polarity.

#### V. Anatomical identification of cone pathways:

This project is in collaboration with Dr. S.J. Schein and aims to the anatomical identification of neurons receiving input from a given type of cone mechanism, by taking advantage of the selective intracellular accumulation of  $C^{14}$ -deoxy-2-glucose in active neurons.

Our approach has been to stimulate with appropriate chromatic lights macaques injected with the radionuclide. After a period of time, the animals are killed and their brains are removed and rapidly frozen with liquid nitrogen. Histological sections are then cut and processed for autoradiography. Preliminary experiments indicate the feasibility of this approach and systematic studies are planned for the coming year. We are also examining the possibility of using a higher resolution technique that will permit us to identify single cells in selected visual regions (e.g. retina and layer four of the striate cortex).

#### Significance to Biomedical Research and the Program of the Institute:

Understanding the organization of the visual system of rhesus and cynomolgus monkeys is very valuable for understanding the human visual system, which at present can only be studied by indirect methods. Radionuclide-label studies appear to be one of the most promising approaches in this direction, because autoradiographic studies can be substituted by non-invasive methods of mapping the distribution of a (gamma-emitter) radionuclide.

Proposed Course: To continue the intracellular retinal and the autoradiographic studies, in addition to more conventional electrophysiological studies of the visual cortex.

NEI Research Program: Retinal and Choroidal Diseases--Retinal Organization and Visual Adaptation; Sensory and Motor Disorders of Vision--Visual Sensory and Perceptual Disorders

Publications:

de Monasterio FM, Gouras P: Responses of macaque ganglion cells to far violet lights. Vision Res 17:1147-1156, 1977.

de Monasterio FM: Spectral interactions in horizontal and ganglion cells of the isolated and arterially-perfused rabbit retina. Brain Res (in press).

de Monasterio FM: Macular pigmentation and the spectral sensitivity of retinal ganglion cells of macaques. Vision Res (in press).

de Monasterio FM: Properties of concentrically-organized X and Y ganglion cells of the macaque retina. J Neurophysiol (in press).

de Monasterio FM: Center and surround mechanisms of opponent-color X and Y ganglion cells of the retina of macaques. J Neurophysiol (in press).

de Monasterio FM: Properties of ganglion cells with atypical receptive-field organization in the retina of macaques. J Neurophysiol (in press).

de Monasterio FM: Asymmetry of ON- and OFF-pathways of blue-sensitive cones of the retina of macaques. Brain Res (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00026-07 LVR
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PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Physiology of the Primate Visual System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Peter Gouras	M.D.	Head, Section on Physiology	LVR NEI
Other:	Eberhart Zrenner	M.D.	Fogarty International Fellow	LVR NEI
	Ralph Nelson	Ph.D.	Senior Staff Fellow	LVR NEI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Vision Research

SECTION

Section on Neurophysiology

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

1.5

PROFESSIONAL:

1.5

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS

☐ (b) HUMAN TISSUES

☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is designed to study the functional properties of single neurons in the retina of the rhesus monkey by extra- and intra-cellular recording techniques. In particular, the spatial, temporal, and chromatic properties of single ganglion cells and S-potentials are being studied. Selected cells, which have been studied physiologically, are injected intracellularly with stains in order to identify them by light and electron microscopy.

Project Description:

Objectives: To understand the cellular basis of vision in a primate similar to man.

Methods Employed: Electrophysiological recordings from single neurons in the retina and visual cortex of anesthetized, paralyzed macaque monkeys; correlation of the distribution of single cell varieties and morphological cell types as seen by electron and light microscopy; the use of refined optical stimuli to define quantitatively spatial, temporal, and chromatic properties of these neurons.

Major Findings: Color opponent ganglion cells lose their color opponency at high flicker frequencies. This is due to frequency-dependent phase shift between center and surround mechanisms in the receptive fields of such cells. It enables these cells to utilize color contrast best at low and luminance contrast best at high flicker frequencies. It undoubtedly explains the curious subjective phenomenon that color flicker is better at low and luminance flicker better at high frequencies.

Red- and green-sensitive cone mechanisms have higher flicker fusion frequencies than the blue-sensitive cone mechanism.

The blue-sensitive cone mechanism shows some unique functional properties at the ganglion cell level: large receptive field size, saturation, transient tritanopia, surround escape, and almost exclusively on-center channels.

The blue-sensitive cone mechanism appears unable to influence a large fraction of S-potentials in monkey retina.

Intracellular recordings from and dye-injection of single ganglion cells and S-potentials are technically feasible in monkey retina.

Significance to Biomedical Research and the Program of the Institute: It is important to understand the physiology of the primate retina in order to explain the cellular basis of human vision and its interruption by various disease states.

Proposed Course: The project is being terminated because the P.I. is leaving NIH to accept a position at Columbia University in New York City.

NEI Research Program: Retinal and Choroidal Diseases--Retinal Organization and Visual Adaptation

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00064-01 LVR
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PERIOD COVERED  
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)  
  
Functional Anatomy of Primate Retina

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Avery Dickinson Nelson	Ph.D.	Staff Fellow	LVR	NEI
Other:	Peter Gouras	M.D.	Head, Section on Neurophysiology	LVR	NEI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Vision Research

SECTION

Section on Neurophysiology

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The long-range objective of this project is to understand the functional organization of primate retina. Our present experiments on rhesus monkey are concerned with (1) identifying the ultrastructural characteristics of different types of photoreceptors and of photoreceptors in different areas of retina which may correlate with the electrophysiological responses of these cells; (2) examining light/dark differences in activity of photoreceptor terminals with the electron microscope, as measured by uptake of the enzyme tracer horseradish peroxidase into synaptic vesicles; (3) characterizing light/dark ultrastructural changes within photoreceptor terminals; (4) identifying specific photoreceptor systems using horseradish peroxidase uptake into synaptic vesicles and selective chromatic adaptation.

Project Description:

Objectives: To understand the functional organization of primate retina.

Methods Employed: Horseradish peroxidase, an enzyme tracer, was injected into the vitreous bodies of anesthetized rhesus monkeys while the retinas were exposed to selective chromatic stimuli. Retinas were fixed by intravitreal injection of aldehydes, reacted with appropriate substrates to form an electron-dense reaction product, and processed for electron microscopy. One-half and one micron sections were examined with the light microscope; ultrathin sections were examined with the electron microscope.

Major Findings: Horseradish peroxidase penetrated extracellular space throughout the retina to the pigment epithelium within three hours of intravitreal injection. Uptake of peroxidase into synaptic vesicles of receptor terminals was found both in eyes exposed to light and in eyes exposed to dark. We are in the process of determining the total numbers of synaptic vesicles per section of terminal and per unit area, the numbers of peroxidase-loaded synaptic vesicles per section of terminal and per unit area, and the percentage of synaptic vesicles containing peroxidase. Preliminary findings suggest that there is a small light/dark difference in the direction of increased loading in the dark. Peroxidase was also found within cone outer segments but not within rod outer segments. It adhered to electron-dense amorphous material which characteristically surrounds cone outer segments and lies in patches adjacent to rod outer segments.

Several structural differences between photoreceptors have become apparent in these studies. We have found two morphologically distinct classes of cone terminals. The first is commonly seen throughout the retina; the second, characterized by greater concentrations of synaptic vesicles and more dense axoplasm, is seen less often and is excluded from the rod-free area of the foveal pit. We anticipate that these classes of terminals may relate to the different cone mechanisms found physiologically. We also noted with interest in transverse sections of Henle's layer some intermediate sized fibers in addition to large diameter fibers which could be attributed to cones and small diameter fibers which could be attributed to rods. In longitudinal section these were identified as varicosities in the rod fibers. The function of these varicosities remains to be determined.

Significance to Biomedical Research and the Program of the Institute: Understanding visual functioning at the cellular level in rhesus monkey retina is valuable in understanding human visual functioning and malfunctioning, which for many retinal diseases has its origins at the cellular level. The foveal region, the focus of our current observations, provides major visual input without which man is legally blind.

Proposed Course: To continue the current experiments.

NEI Research Program: Retinal and Choroidal Diseases--Visual Cells and Pigment Epithelium

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00005-06 LVR
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PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Electrophysiological Studies of Mammalian Retina

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Ralph Nelson	Ph.D.	Staff Fellow	LVR	NEI
	Peter Gouras	M.D.	Head, Section on Neurophysiology	LVR	NEI
	Eberhart Zrenner	M.D.	Fogarty Fellow	LVR	NEI

COOPERATING UNITS (if any)

Helga Kolb, Ph.D., Laboratory of Neurophysiology, NINCDS

LAB/BRANCH

Laboratory of Vision Research

SECTION

Section on Neurophysiology

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

1.7

PROFESSIONAL:

1.7

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS      ☐ (b) HUMAN TISSUES      ☒ (c) NEITHER

☐ (a1) MINORS    ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The goal of this project is to obtain information about the normal functioning of mammalian retinas. We study single neurons in the cat retina, investigate neural responses to photic stimuli using intracellular recording, and characterize neural morphology with intracellular injections of stains. A new, enzymatic microelectrode stain, horseradish peroxidase (HRP), produces a dense reaction product visible in the electron microscope. With this we have observed the synapses impinging on single, electrophysiologically studied retinal neurons. A new physiological class of amacrine cell, the AIII amacrine cell, has hyperpolarizing responses similar to those of horizontal cells. The HRP stain reveals that the synaptic input to these cells originates with rod bipolar and invaginating cone bipolar axon terminals and with one or more classes of other amacrine cells. We suspect the AIII amacrine may provide signals contributing to surround mechanisms operating in the inner plexiform layer (IPL). Patterned stimuli were constructed to examine the receptive field properties of the electroretinogram (ERG) and to relate these properties to those of single neurons. Results indicate that the ERG b-wave responses of the cat retina may also originate in part with surround mechanisms operating in the IPL.

Project Description:

Objectives: To understand the functional and structural organization of mammalian retinas, to elucidate the neural circuitry and functional pathways in these retinas, and to relate this organization to disease states.

Methods Employed: We characterize the response properties of neurons, principally in the cat retina, by intracellular and extracellular recording of their electrical activity during photic stimulation and by electroretinography. We maintain viable retina-eyecup preparations in vitro using arterial perfusion of the eye with synthetic media. HRP, injected into neurons through the electrodes, fills their axons and dendrites and, after incubation with appropriate reagents, reveals neuronal morphology in the light microscope and the details of synaptic input and output in the electron microscope. We relate such anatomical inputs to those found electrophysiologically. Inputs from the rod and cone systems are assessed principally by comparison of responses to matched monochromatic stimuli, under conditions of red, yellow, blue, or dark adaptation. We measure receptive field sizes from the responses to long narrow slits placed different distances from the receptive field centers; from this we compute space constants. These receptive fields are compared to the anatomical dendritic fields of the neurons studied to indicate the extent of lateral interactions.

Major Findings:

## I. Properties of AII and AIII amacrine cells in the cat retina:

Two classes of amacrine cells with relatively small dendritic fields (60-90  $\mu\text{m}$ ) have been studied in the cat retina and have been found to have remarkably disparate properties. The first (AII) had been previously described anatomically with the classical Golgi and serial-section electron microscopic techniques. This cell receives chemical synaptic input from the ribbon synapses of the rod bipolar cell axon terminals; it is interconnected with the axon terminals of invaginating cone bipolar cells through gap junctions, and it provides output to several classes of neurons in the off-layer (sublamina a) of the IPL. Physiologically such amacrine cells have responses resembling those of bipolar cells described in other species. The initial response to suprathreshold, centered, light stimuli is a sharp, saw toothed transient followed by a slow sag; the potential sometimes returns all the way back to the original resting level. At mesopic intensities of stimulation a slower hyperpolarization of the membrane follows the "stimulus off." When stimuli are moved just slightly away from the center, an initial hyperpolarization or antagonistic surround can often be seen. The region of center responses, perhaps owing to this surround antagonism, is extremely small and is characterized by space constants of only 20 to 70  $\mu\text{m}$  (an edge-to-edge width for 1/e attenuation of the peak response of 40 to 140  $\mu\text{m}$ ). No smaller center sizes have been found in the cat retina. These AII units appear to be dominated by chemical synaptic input from the rod bipolar cells, since they are more sensitive than most neurons in cat retina, and since they are nearly univariant in response to rod-matched stimuli. They are characterized in the dark by a good deal of baseline noise; this is quieted by light. Stimulus sensitive noise can originate at many levels of retinal

processing; however, since even threshold stimuli appear to reduce noise, we argue that photon/thermal isomerization noise is not involved since additional photons should increase the variance of this source. One possibility is that the reduction in noise is related to a decrease in the rate of vesicle release by a hyperpolarizing presynaptic element such as the rod bipolar. The small concentric receptive field organization of the AII amacrine implies that it may be an important source of signals for the center mechanisms of ganglion cells and other IPL neurons.

We first discovered the other small field amacrine cell, the AIII unit, using the microelectrode, HRP-injection technique. This unit, like the AII, receives ribbon synaptic input from the axon terminals of rod bipolar cells. Unlike the AII amacrine, however, the AIII also receives such input from the axonal terminals of invaginating cone bipolar terminals and has extensive input from other amacrine cells. The HRP staining has tended to obscure the cytoplasmic markers for synaptic output for this cell. Physiologically, the AIII amacrine has properties just opposite to those of the AII. Its response to a centered stimulus is a maintained hyperpolarization. The receptive field center is extremely broad, having a space constant of about 200 microns (1/e width, edge to edge, of 400  $\mu$ m). There is little or no baseline noise and only just perceptible quieting of such noise during light. These units are physiologically difficult to distinguish from horizontal cell responses recorded in the outer plexiform layer of the cat retina. The maximum amplitudes of AIII responses are somewhat smaller than those of horizontal cells, 10 to 20 mV as compared to 20 to 40 mV. Although having more cone signals than the AII amacrine (perhaps a reflection of the additional chemical synaptic input from invaginating cone bipolar axon terminals). AIII amacrine have somewhat less cone input than found in the responses of horizontal cell bodies, perhaps 20 to 30% compared to 50 to 80% for horizontal cell bodies. Owing to their broad field and relatively high thresholds (compared to AII cells) these AIII units appear to be a likely source of signals contributing to surround mechanisms operating in the IPL.

## II. Electroretinographic responses to patterned stimuli:

Noting that many neurons in the retinas of cats and other vertebrate species have concentric center-surround organization of their receptive fields, we decided to design whole-retina stimuli that might test for center-surround organization in the electroretinogram. Two sorts of patterns were conceived. One consisted of hexagonally arrayed spots, the other of parallel bars. Since, from our experience with single units, we were aware that receptive field surrounds tended to be much larger than receptive field centers, we took care to separate the spots in our multispot stimuli (or bars in the striped patterns) by distances much larger than their diameters (or widths of the bars in the striped patterns). A factor of 7 for spot separation/diameter and 11 for bar width/separation was employed. Keeping these factors constant, we generated a series of whole retina patterns with varying spot diameters (or bar widths). A feature of these stimulus patterns is that equal retinal areas are always illuminated so that the ERG responses were always generated by the same net stimulus area, albeit differently arranged on the retinal surface. Quantal

flux per unit area in the illuminated regions was also kept constant. For other reasons we anticipated that the ERG b-wave might reflect the center mechanisms of the cat retina and that we would find an optimal spot separation that would give us enhanced, maximal b-waves without interference from surrounds. Experimental evidence disabused us of this expectation. As the spot diameters and spot separations in these patterns were increased, b-wave responses diminished precipitously and disappeared altogether when a pattern of 250  $\mu\text{m}$  spots separated by 1.6 mm was used. With further increases in spot diameter, the b-wave reappeared. A similar minimum at 300  $\mu\text{m}$  widths was noted for the bar patterns. A model has been developed which suggests that these results are more consistent with the notion that the b-wave may be involved in the surround mechanism of the cat retina and that the ERG a-wave responses may reflect the activity of center mechanisms.

The anatomical aspects of this project are described in Project No. Z01 NS 02152-04 LNP, NINCDS; Dr. Helga Kolb, principal investigator.

Significance to Biomedical Research and the Program of the Institute: In diagnosing and treating the diseases of the eye it should prove useful to understand retinal function at the cellular level. Many disease states involve dysfunction at the cellular level and treatments have as their targets particular classes of cells. A knowledge of what classes of neurons the retina contains, and what their physiological properties and role in vision may be, provides a necessary substrate for interpreting and treating retinal dysfunction.

Proposed Course: This project will be continued with particular emphasis on the HRP staining technique to elucidate further the interconnections, pathways, and physiological properties of neurons participating in the IPL.

NEI Research Program: Retinal and Choroidal Diseases--Retinal Organization and Visual Adaption

#### Publications:

Nelson R, Famiglietti EV Jr, Kolb H: Intracellular staining reveals different levels of stratification for on- and off-center ganglion cells in cat retina. J Neurophysiol 41:472-483, 1978.

Nelson R, Zrenner EZ, Gouras P: Patterned stimuli reveal spatial organization in the electroretinogram. Jpn J Ophthalmol (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE  
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF  
HEALTH, EDUCATION, AND WELFARE  
PUBLIC HEALTH SERVICE  
NOTICE OF  
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00066-01 LVR

PERIOD COVERED

January 3, 1978 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Neurotransmitter Chemistry of Retinal Neurons

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER  
PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Barbara-Anne Battelle Ph.D. Staff Fellow LVR NEI

Other: None

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Vision Research

SECTION

Section on Retinal and Corneal Metabolism

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

0.6

PROFESSIONAL:

0.6

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS

☐ (b) HUMAN TISSUES

☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies are underway to identify neurotransmitters in photoreceptor cells and other retinal neurons. Two systems are currently being investigated: (1) Limulus photoreceptors and (2) human retinoblastoma cells. Experiments to date have revealed that Limulus visual cells actively synthesize octopamine and an unknown metabolite. Retinoblastoma cells synthesize small quantities of acetylcholine.

Project Description:

Objectives: In order to understand the processing of visual information in retinas, it is necessary to know the morphological, electrophysiological, and neurochemical organization of retinal neurons. The morphological organization and electrophysiological properties of retinal cells have been studied extensively and are in part understood. The neurotransmitter chemistry of retinal cells is largely unknown. The general aim of my research is to determine the neurotransmitters used by identified retinal neurons and to examine neurochemical mechanisms involved in processing visual information.

Two projects have been started since this laboratory was set up: (1) An examination of neurotransmitters in Limulus visual cells; (2) a study of possible neuronal properties in human retinoblastoma cells.

Methods Employed: Biochemical methods are used to study neurotransmitter synthesis. Retinoblastoma cells or Limulus eyes are incubated in vitro with radioactively labelled precursors of potential neurotransmitters. After the incubation, an acid soluble extract of the tissue is subjected to high voltage paper electrophoresis, a chromatographic procedure that allows for rapid and effective separation of radioactive precursor substances from their products. The amount of presumptive neurotransmitter synthesized by tissues is quantified using liquid scintillation counting.

Major Findings: (1) Neurotransmitters in Limulus visual cells: Results of experiments I performed before arriving here in January suggested that octopamine (the phenol analogue of norepinephrine) might be the neurotransmitter used by photoreceptor cells of the Limulus ventral eye. Ventral eye photoreceptors incubated with tyrosine or tyramine synthesized octopamine as well as large quantities of an unknown metabolite. They did not synthesize dopamine or norepinephrine from tyrosine, nor did they synthesize GABA from glutamate or acetylcholine from choline. These initial observations have been confirmed in more recent experiments. The nature of the unknown metabolite is being investigated, and results of preliminary experiments suggest it is a derivative of octopamine and tyramine. When the metabolite is subjected to mild acid hydrolysis, it is destroyed and the label is recovered as octopamine and tyramine.

Preparations of Limulus lateral and medial eyes incubated with tyrosine or tyramine also synthesize octopamine and the unknown metabolite. Ventral, medial and lateral eye preparations incubated with tryptophan form two major radioactive products neither of which are serotonin. The identity of these products is as yet unknown, but they are likely to be tryptamine and a tryptamine metabolite. Failure to observe serotonin synthesis in any of the Limulus eyes is of interest in view of suggestions made by other investigators that serotonin can modulate photoreceptor cell sensitivity in Limulus lateral eyes. It may be that tryptamine and not serotonin is the endogenous modulator.

(2) Possible neuronal properties of human retinoblastoma cells: Retinoblastoma cells have been assayed for amine, serotonin, GABA and acetylcholine synthesis. They do not synthesize GABA, serotonin or any of the amines, however

they do synthesize small quantities of acetylcholine. The identity of the choline product was verified by hydrolysis with specific acetylcholinesterase.

Significance to Biomedical Research and the Program of the Institute:

Knowledge of neurotransmitters in retinas is vital to the understanding of how visual information is processed. The identity of the neurotransmitters used by photoreceptor cells of vertebrate and invertebrate eyes is completely unknown. Limulus eyes are relatively simple and are composed principally of photoreceptor cells. Because of this simplicity the likelihood is high for determining conclusively the identity of Limulus photoreceptor cell neurotransmitters. By studying the relatively simple invertebrate preparations, it is hoped that insights will be gained into the neurochemical properties of photoreceptor cells from more complex vertebrate eyes.

The cellular origins of retinoblastoma are unknown. If one of the retinoblastomas turns out to be a uniform population of transformed retinal neurons, it could prove extremely useful in the study of the neurochemistry of retinal cells. A study of the biochemical properties of retinoblastoma will also increase the likelihood of finding an effective method of controlling the growth of retinoblastomas.

Proposed Course: Studies of neurotransmitter synthesis in Limulus ventral, lateral, and medial eyes will continue. Considerable effort will be directed toward the identification of the unknown metabolite synthesized from tyrosine and tyramine, and experiments will be done to test if octopamine or the metabolite can be released from photoreceptor cells with light or high potassium stimulation.

The characterization of biochemical properties of retinoblastoma cells will continue with a series of amino acid and neurotransmitter uptake experiments. A search for specific cell surface receptors will be started with assays for amine-stimulated adenylate cyclase activity.

NEI Research Program: Retinal and Choroidal Diseases--Retinal Organization and Visual Adaptation

Publications:

Battelle BA, Kravity EA: Targets of octopamine action in the lobster: Cyclic nucleotide changes and physiological effects in hemolymph heart and ~~ekoskeletal~~ muscle. J Pharmacol Exp Ther 205:438-448, 1978.

Battelle BA, LaVail MM: Rhodopsin content and rod outer segment length in albino rat eyes: Modification by dark adaptation. Exp Eye Res 26:487-497, 1978.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  201 EY 00148-05 LVR
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PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Cyclic Nucleotides and Vision

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Gerald J. Chader	Ph.D.	Research Chemist	LVR NEI
Other:	R. Theodore Fletcher	M.S.	Chemist	LVR NEI

COOPERATING UNITS (if any)

1) Gopal Krishna, Ph.D., Lab. Chem. Pharmacol, NHLBI, 2) Gustavo Aguirre, D.V.M., Dept. Ophthalmol., Sch. Vet. Med., U. of Pa., Phila., Pa., 3) Dr. Richard Lolley and Dr. Debora Farber, VA Hospital, Sepulveda, Ca.

LAB/BRANCH

Laboratory of Vision Research

SECTION

Section on Retinal and Corneal Metabolism

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

1.4

PROFESSIONAL:

0.4

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS

☐ (b) HUMAN TISSUES

☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Cyclic nucleotides, especially cyclic GMP, appear to be important in retina and pigment epithelium. This is true both in adult and embryonic tissue. Guanylate cyclase, the enzyme that catalyzes the formation of cyclic GMP, is in extremely high concentration in the neural retina and has several unique properties. Phosphodiesterase, the enzyme that metabolizes cyclic GMP, demonstrates very low activity in retinas from dogs with retinal degeneration, leading us to postulate that resultant abnormally high cyclic GMP concentrations are probably intimately involved in the disease process.

Project Description:

Objectives: To study the role of cyclic nucleotides in normal vision and in retinal diseases.

Methods Employed: Retinas from test animals are dissected by standard techniques; photoreceptors are isolated by sucrose density gradient centrifugation, and the activities of the enzymes of cyclic nucleotide metabolism are assayed by standard techniques. Cyclic nucleotide concentrations are measured by immunochemical titration after initial purification by column chromatography.

Major Findings: We have studied the development of the enzymes of cyclic nucleotide metabolism in embryonic chick retina and pigment epithelium. In retina, both cyclic AMP and cyclic GMP increase during embryonic development, whereas cyclic AMP decreases in pigment epithelium. Cyclic AMP-dependent protein kinase activity is present in embryonic retina as well as active GTP-protein kinase activity.

Guanylate cyclase is present in very high concentration in bovine retinas. It is almost wholly membrane-bound and is substantially inhibited by calcium ion. In the presence of manganese, the enzyme exhibits strong negative cooperativity.

Phosphodiesterase activity is very low in retinas of dogs (Irish setters) afflicted with retinal degeneration. Cyclic GMP concentrations are extremely high even in quite young animals, leading us to postulate that this may represent the primary genetic lesion in this heredity disease.

Significance to Biomedical Research and the Program of the Institute: Study of the enzymes of cyclic nucleotide synthesis and degradation gives a better understanding of how the normal retina functions and could uncover the basic cause of at least one form of retinal degeneration.

Proposed Course: We will continue our studies using both normal and diseased ocular tissues.

NEI Research Program: Retinal and Choroidal Diseases--Developmental and Hereditary Disorders

Publications:

Krishnan N, Fletcher R, Chader G, Krishna G: Characterization of guanylate cyclase of rod outer segments of the bovine retina. Biochim Biophys Acta 523:506-515, 1978.

Aguirre G, Farber D, Lolley R, Fletcher RT, Chader GJ: Rod-cone dysplasia in Irish setter dogs: A defect in cyclic GMP metabolism of visual cells. Science (in press).

Fletcher RT, Chader GJ: Cyclic nucleotides and protein kinase systems in the developing chick retina and pigment epithelium. Biochim Biophys Acta (in press).

Tamai M, Teirstein P, Goldman A, O'Brien P, Chader GJ: The pineal gland does not control rod outer segment shedding in the rat retina and pigment epithelium. Invest Ophthalmol Visual Sci 17:558-562, 1978.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRANURAL RESEARCH PROJECT	PROJECT NUMBER  <div style="text-align: right;">Z01 EY 00179-03 LVR</div>																								
PERIOD COVERED <div style="text-align: center;">October 1, 1977 to September 30, 1978</div>																										
TITLE OF PROJECT (80 characters or less)  <div style="text-align: center;">Ultrastructural and Biochemical Correlates in the Vertebrate Retina</div>																										
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																										
<table style="width:100%; border: none;"> <tr> <td style="width:15%;">PI:</td> <td style="width:30%;">Arnold I. Goldman</td> <td style="width:15%;">Ph.D.</td> <td style="width:20%;">Staff Fellow</td> <td style="width:20%; text-align: right;">LVR NEI</td> </tr> <tr> <td rowspan="2">Other:</td> <td>Paul O'Brien</td> <td>Ph.D.</td> <td>Research Biochemist</td> <td style="text-align: right;">LVR NEI</td> </tr> <tr> <td>Paul S. Teirstein</td> <td>B.S.</td> <td>Medical Student</td> <td style="text-align: right;">Mount Sinai Hospital and Medical School</td> </tr> <tr> <td></td> <td>Makoto Tamai</td> <td>Ph.D.</td> <td>Visiting Scientist</td> <td style="text-align: right;">LVR NEI</td> </tr> <tr> <td></td> <td>Gerald Chader</td> <td>Ph.D.</td> <td>Head, Section on Retinal and Corneal Metabolism</td> <td style="text-align: right;">LVR NEI</td> </tr> </table>			PI:	Arnold I. Goldman	Ph.D.	Staff Fellow	LVR NEI	Other:	Paul O'Brien	Ph.D.	Research Biochemist	LVR NEI	Paul S. Teirstein	B.S.	Medical Student	Mount Sinai Hospital and Medical School		Makoto Tamai	Ph.D.	Visiting Scientist	LVR NEI		Gerald Chader	Ph.D.	Head, Section on Retinal and Corneal Metabolism	LVR NEI
PI:	Arnold I. Goldman	Ph.D.	Staff Fellow	LVR NEI																						
Other:	Paul O'Brien	Ph.D.	Research Biochemist	LVR NEI																						
	Paul S. Teirstein	B.S.	Medical Student	Mount Sinai Hospital and Medical School																						
	Makoto Tamai	Ph.D.	Visiting Scientist	LVR NEI																						
	Gerald Chader	Ph.D.	Head, Section on Retinal and Corneal Metabolism	LVR NEI																						
COOPERATING UNITS (if any)  <div style="text-align: center;">Mount Sinai Hospital and Medical School</div>																										
LAB/BRANCH <div style="text-align: center;">Laboratory of Vision Research</div>																										
SECTION <div style="text-align: center;">Section on Retinal and Corneal Metabolism</div>																										
INSTITUTE AND LOCATION <div style="text-align: center;">National Eye Institute, NIH, Bethesda, Maryland 20014</div>																										
TOTAL MANYEARS: <div style="text-align: center;">2.0</div>	PROFESSIONAL: <div style="text-align: center;">1.2</div>	OTHER: <div style="text-align: center;">0.8</div>																								
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div> <input type="checkbox"/> (a) HUMAN SUBJECTS         </div> <div> <input type="checkbox"/> (b) HUMAN TISSUES         </div> <div> <input checked="" type="checkbox"/> (c) NEITHER         </div> </div> <div style="margin-top: 10px;"> <input type="checkbox"/> (a1) MINORS    <input type="checkbox"/> (a2) INTERVIEWS       </div>																										
SUMMARY OF WORK (200 words or less - underline keywords)  <p>         Studies are being conducted on the processes of <u>shedding of outer segment membranes</u> and their subsequent <u>phagocytosis</u> by the <u>pigment epithelium</u> with emphasis on <u>control mechanisms</u> of these processes. <u>Phagosome counts by light microscopy</u> and <u>autoradiography</u> are the major analytical techniques, although <u>electron microscopy</u> is used when appropriate. The <u>shedding rhythm of albino rats</u> persisted after five days in <u>constant darkness</u> but was abolished by exposure to <u>constant light</u>. <u>Pinealectomy</u>, <u>superior cervical ganglionectomy</u>, and <u>hypophysectomy</u> had no effect on the rhythm, although injections of <u>melatonin</u> and <u>isoproteranol</u> could stimulate shedding. <u>Serotonin N-acetyltransferase activity</u> was detected in the <u>retina</u>, although no diurnal rhythm was evident. Five days after animals were placed on a <u>shifted lighting schedule</u>, a new pattern of shedding was observed. If one eye was <u>occluded</u> and the rat was placed in altered <u>lighting</u>, the closed eye maintained the original rhythm, while the open eye responded to the new lighting conditions. <u>RCS rats</u> were found to have a small peak of phagocytosis one hour after the onset of light.       </p>																										

Project Description:

Objectives: It has been established that animals which have been maintained under conditions of cyclic lighting exhibit a daily burst of shedding and phagocytosis of outer segment tips within two hours of the onset of light. This occurs on schedule even if animals are placed in constant darkness after entrainment. The experiments in this project were designed to search for the site of control of this phenomenon and the mechanisms by which this control is exercised. By examining patterns of phagocytosis in the pigment epithelium of the RCS rat, a strain which has a defect in phagocytosis, it is hoped that the details of this defect can be determined, leading to better understanding of the mechanisms of phagocytosis in both normal and disease conditions.

Methods Employed: Light microscopy was used to observe patterns of shedding and phagocytosis. Radiobiochemical analysis was used to determine serotonin N-acetyltransferase activity of the retina. Animals were treated by modification of lighting schedules, surgical ablation of suspected circadian pacemakers, and injection of hormones thought to transmit information about circadian rhythms.

Major Findings: None of the surgical procedures produced an alteration in the pattern of shedding and phagocytosis. The presence of serotonin N-acetyltransferase activity in the retina and the fact that animals injected with melatonin or isoprotornol while in constant light exhibited a burst of shedding indicate that melatonin or its analog may be involved with the control of shedding at the retinal level. Rats kept in constant darkness five days maintained the shedding rhythm, but 24 hours exposure to constant light was sufficient to block this response. A dark period of as short as two hours is sufficient to prepare the retina for shedding. Five days after placing animals on a shifted lighting schedule, a new pattern of shedding was observed. When one eye of a rat was occluded and the animal was placed under new lighting conditions, the covered eye maintained the original shedding rhythm, while the open eye adjusted to the new lighting conditions. RCS rats were found to exhibit a peak of phagocytosis one hour after light onset, although at levels about 5% of those of the control animals. When eyecups of either RCS or normal rats were incubated in organ culture for three hours a large burst of shedding and/or phagocytosis was observed, at a level of between seven and ten times baseline.

Significance to Biomedical Research and the Program of the Institute: The mechanisms of regulation of shedding of discs and their subsequent phagocytosis by the pigment epithelium are crucial in the understanding of photoreceptor renewal processes. By learning which organ(s) control the process of shedding and phagocytosis and the biochemical triggers for these events, it may be possible to develop new treatments for various retinal diseases in which the balance between synthesis and degradation of photoreceptor membranes is disturbed.

Proposed Course: Studies of the effect of varying lighting schedules will be intensified. Specifically, the dark period will be shortened until the shedding rhythm can no longer be maintained. Extended periods of darkness will be used to test the circadian nature of the rhythm and to see whether it dies

out in time. Experiments will be initiated to determine the most effective method of changing the rhythm in the minimum of time. Some of these tests will be repeated in the RCS rat to see if the rhythm in that animal responds in the same way as in control animals. In addition, autoradiographic studies will be used with intravitreal injections of sugars in an attempt to find membrane changes associated with outer segment shedding.

NEI Research Program: Retinal and Choroidal Diseases--Visual Cells and Pigment Epithelium

Publications:

Goldman AI, O'Brien PJ: Phagocytosis in the retinal pigment epithelium of the RCS rat. Science (in press).

Tamai M, Teirstein P, Goldman A, O'Brien P, Chader G: The pineal gland does not control shedding and phagocytosis in the rat retina and pigment epithelium. Invest Ophthalmol Visual Sci 17:558-562, 1978.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00068-01 LVR
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PERIOD COVERED

October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)

Physiology of the Pigment Epithelium

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Eileen Masterson	Ph.D.	Postdoctoral Fellow	LVR	NEI
Other:	Paul Israel		Biological Aide	LVR	NEI
	Gerald J. Chader	Ph.D.	Research Chemist	LVR	NEI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Vision Research

SECTION

Section on Retinal and Corneal Metabolism

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

1.0

PROFESSIONAL:

0.8

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Intact pigment epithelium from chick embryos has no appreciable pentose phosphate pathway activity. In contrast, cultured pigment epithelial cells have a significant amount of activity, indicating that cultured cells metabolize in a way very different from those in vivo. Intact retinas from chick embryos demonstrate a decline in activity of this pathway during development. Cultured retinal cells remain generally undifferentiated in tissue culture and resemble the blast cells from early stages.

Project Description:

Objectives: To study the normal development of retina and pigment epithelium in vivo and in culture.

Methods Employed: Pentose pathway activity is studied using radiolabelled glucose, either carbon-1 or carbon-6 labelled. Intracellular water content in these cultured cells is measured using radiolabelled 3-O-methylglucose, and the transport of various radiolabelled sugars and amino acids by these cells is also evaluated using standard techniques.

Major Findings: Pentose shunt activity in developing chick retina and pigment epithelium was studied by measuring the rate of  $^{14}\text{CO}_2$  evolution from glucose selectively labelled in the C-1 and C-6 positions. In developing retina shunt activity declines from relatively high levels at stages 29-31 to minimal activity in the two-week old chick. Overall retinal metabolism also declines up to stages 45 but dramatically increases again after hatching. Cultured chick neural retina retains a high shunt activity although metabolic activity is low. Developing pigment epithelium has minimal shunt activity at all stages studied. In contrast, cultured chick pigment epithelium has appreciable shunt activity which is constant over a period of several weeks in culture. Differences in metabolic activity between the intact and cultured situation should be taken into account in biochemical studies of these tissues in culture. The correlation of high shunt activity with active cell proliferation in both intact and cultured tissues indicates a prominent role for this metabolic pathway in normal retinal development. It may also play a role in abnormal retinal development and pigment epithelium development. Changes in the biochemical functioning of pigment epithelial cells in culture may prelude morphological changes seen in long term culture of this tissue, i.e. lentoid bodies.

Studies of transport functions in cultured pigment epithelium have demonstrated that these cells will take up 3-O-methylglucose with time until an equilibrium is established whereby the intracellular concentration of this hexose equals the extracellular concentration. Acceleration exchange diffusion of this sugar was also demonstrated. The efflux of 3-O-methylglucose from the cells was inhibited by phloretin, a potent inhibitor of sugar transport in eukaryotic cells. The energy poisons sodium azide and dinitrophenol had no effect on 3-O-methylglucose uptake by cultured chick pigment epithelium, indicating this is a facilitated diffusion system. In contrast, the uptake of the nonmetabolizable amino acid  $\alpha$ -aminoisobutyric acid is inhibited by these compounds, demonstrating the active transport of this amino acid by the cultured cells. Finally, studies of the transport of D- and L-glucose by cultured chick pigment epithelial cells have demonstrated a stereo-specific carrier for D-glucose in these cells.

Significance to Biomedical Research and the Program of the Institute: A comprehensive study of developing pigment epithelium and its metabolic and transport functions would serve several purposes. Such a study may lead to a better understanding of certain retinal-pigment epithelium diseases such as retinitis pigmentosa. The study of pigment epithelial metabolism during devel

opment may also help in our understanding of normal retinal-pigment epithelium interactions.

Proposed Course: This project will be continued. We plan to evaluate the effects of various hormones such as insulin and the effect of various drugs and other agents such as sulfhydryl reagents on metabolism and transport functions of the pigment epithelium. By examining the effects these external agents have, we may better understand the response of these cells to a variety of chemical messengers.

NEI Research Program: Retinal and Choroidal Diseases--Developmental and Hereditary Disorders

Publications:

Masterson E, Israel P, Chader GJ: Pentose shunt activity in developing and cultured retina and pigment epithelium. A switch in biochemical expression in cultures of pigment epithelial cells. Exp Eye Res (in press).

Israel P, Redfern N, Chader GJ: Neural retinal-pigment epithelial interactions; Morphological characteristics of cells grown together in culture. Ophthal Res (in press).



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE <b>NOTICE OF</b> INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00067-01 LVR
PERIOD COVERED October 1, 1977 to September 30, 1978		
TITLE OF PROJECT (80 characters or less)  Studies on the Developing Cornea		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Other:	Eileen Masterson Paul Israel Paul J. O'Brien David Whikehart Gerald J. Chader	Ph.D.  Ph.D. Ph.D. Ph.D.
	Postdoctoral Fellow Biological Aide Research Chemist Staff Fellow Research Chemist	LVR NEI LVR NEI LVR NEI LVR NEI LVR NEI
COOPERATING UNITS (if any)  None		
LAB/BRANCH Laboratory of Vision Research		
SECTION Section on Retinal and Corneal Metabolism		
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20014		
TOTAL MANYEARS: 0.9	PROFESSIONAL: 0.8	OTHER: 0.1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)  The pattern of <u>glucose oxidation</u> in the <u>developing chick cornea</u> changes with advancing age of the embryo. The <u>pentose phosphate pathway</u> becomes more prominent after <u>transparency development</u> has occurred. <u>Diamide</u> , a compound which oxidizes glutathione in cells, will stimulate the pentose phosphate pathway in the chick cornea at all developmental ages studied.		

Project Description:

Objectives: This study focuses on determining the significance of the various metabolic pathways during corneal development and the fate of glucose incorporated into the cornea during this period. The embryonic chick cornea transforms itself from an opaque tissue to a transparent one; the biochemical phenomena underlying this process are unknown. The data obtained from these studies are aimed at evaluating the basic biochemical processes which occur in the cornea during this critical period of transparency development. Such information may be useful in elucidating the underlying mechanisms of corneal dehydration that are responsible for maintaining clear mammalian corneas. We also feel the chick cornea is a good tissue to use to study the effects of sulfhydryl agents on both clear and opaque corneas.

Methods Employed: Pentose phosphate pathway activity is evaluated using radiolabelled carbon-1 or carbon-6 glucose incubated with corneas in an in vitro system. Incorporation and uptake of various labelled sugars and amino acids are also evaluated in the same system, and the effects of various biological inhibitors and sulfhydryl agents on the corneas' metabolism are studied.

Major Findings: Embryonic chick corneas at different stages of development were evaluated for pentose shunt activity. The appearance of shunt activity was concurrent with the onset of corneal transparency.

Stage 40 to 45 of embryonic development is the critical period of corneal deturgescence and transparency development in the chick embryo. Glucose uptake and incorporation increases between stages 38 and 40, is constant between stages 40 and 45, and increases again only after hatching. The effects of diamide, a relatively specific oxidizer of glutathione, and the general sulfhydryl reagent N-ethylmaleimide (NEM) on the oxidation of the carbon-1 (C-1) and carbon-6 (C-6) atoms of glucose in stages 38 and 45 chick embryonic cornea were studied. Diamide increased both C-1 and C-6 oxidation, but C-1 is preferentially increased, indicating an elevation of pentose phosphate pathway activity with this agent. NEM had the opposite effect, decreasing both C-1, and to a greater extent, C-6 oxidation. Diamide thus appears to increase pentose phosphate pathway activity via its oxidation of glutathione, creating a demand for NADPH within the cell. Results with NEM indicate this agent inhibits the enzymatic machinery of the glycolytic pathway and tricarboxylic acid cycle more effectively than that of the pentose phosphate pathway. Therefore, the pentose pathway is better able to withstand this type of stress than the other glycolytic pathways. It is concluded that the pentose pathway may serve as an important metabolic alternative in the cornea under conditions of metabolic stress.

Significance to Biomedical Research and the Program of the Institute: This project is directed at elucidating some of the basic biochemical events which occur during corneal transparency development in the chick. The maintenance of transparency by the cornea is obviously its most important physiological function. Explorations of the mechanisms which permit the cornea to transform itself into a transparent tissue may aid in the understanding of this process in the adult and of diseases which cause corneal opacity.

Proposed Course: The biochemistry and physiology of corneal transparency will be further probed.

NEI Research Program: Corneal Diseases--Corneal Edema, Dystrophies, and Inherited Disorders

Publications:

Masterson E, Whikehart DR, Chader GJ: Glucose oxidation in the chick cornea: Effect of diamide on the pentose shunt. Invest Ophthalmol Visual Sci 17:449-454, 1978.

Masterson E, Edelhauser HF, Chader GJ: The pentose phosphate pathway in developing chick cornea. Biochim Biophys Acta (in press).



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00016-11 LVR
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PERIOD COVERED  
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)  
The Biochemistry of Normal and Dystrophic Retinas

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Paul J. O'Brien	Ph.D.	Research Chemist	LVR NEI
Other:	Gustavo Aguirre	V.M.D.	Asst. Prof.	U. of PA
	James P. Alligood	B.S.	Biologist	LVR NEI

COOPERATING UNITS (if any)  
School of Veterinary Medicine, University of Pennsylvania

LAB/BRANCH  
Laboratory of Vision Research

SECTION  
Section on Retinal and Corneal Metabolism

INSTITUTE AND LOCATION  
National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 0.8	PROFESSIONAL: 0.4	OTHER: 0.4
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CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS      ☐ (b) HUMAN TISSUES      ☒ (c) NEITHER

☐ (a1) MINORS    ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Gel electrophoresis was used to identify radioactive opsin extracted from the outer segments of dogs injected intravitreally with labeled amino acids. Con-current autoradiography showed a migrating band of labeled opsin in the outer segments of both normal and affected littermates of miniature poodles carrying the gene for an inherited progressive retinal atrophy.

In vitro incubation of bovine, frog, chick and dog retinas with labeled fucose has resulted in the labeling of an opsin-like protein that is distinct from rhodopsin and may be a cone pigment. Thus, fucose may be useful as a marker to follow the viability of cones in degenerating retinas such as in dogs.

Project Description:

Objectives: The renewal of photoreceptor cell outer segments is a continuous process which is impaired in some pathological conditions such as progressive degeneration or developmental anomalies of the retina. The purpose of this project is to elucidate the biochemical events involved in photoreceptor renewal, especially the synthesis of protein, in the retinas of both cow and dog.

Methods Employed: Ordinary biochemical techniques were used, such as incubation of retinas, cell fractionation, isolation of rod outer segments by density gradient centrifugation, detergent extraction and purification of rhodopsin by column chromatography and gel electrophoresis.

Major Findings: Protein synthesis and outer segment renewal was demonstrated in both normal and affected littermates produced by the crossing of an affected and a carrier miniature poodle. Intravitreal injection of radioactive leucine resulted in the production of labeled opsin which was visualized as a migrating band by autoradiography and identified as opsin by gel electrophoresis.

Incubation of bovine, frog, chick, or dog retinas with radioactive fucose produced a labeled protein behaving as opsin on agarose chromatography but not regenerable to rhodopsin upon the addition of retinaldehyde. This protein may represent cone pigments.

Significance to Biomedical Research and the Program of the Institute: No apparent difference in protein synthesis or outer segment renewal could be detected in normal and affected poodles, the latter of which have an inherited progressive retinal atrophy. Thus the defect in this animal model of human retinitis pigmentosa may lie in some other area of photoreceptor metabolism.

The ability to use fucose as a marker for cone pigment synthesis will permit its use in following the viability of cones in retinal degenerative disorders such as was done with the poodles using leucine.

Proposed Course: A series of experiments involving the intravitreal injection of leucine into Irish setters will be undertaken to determine if there is any abnormality in opsin synthesis during the brief time in which outer segments begin to form but before they rapidly degenerate.

NEI Research Program: Retinal and Choroidal Diseases--Developmental and Hereditary Disorders

Publications:

Goldman AI, O'Brien PJ: Phagocytosis in the retinal pigment epithelium of the RCS rat. Science (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00015-13 LVR
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PERIOD COVERED  
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)  
The Cell Biology of the Vertebrate Retina

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Paul J. O'Brien	Ph.D.	Research Chemist	LVR	NEI
Other:	James P. Alligood	B.S.	Biologist	LVR	NEI

COOPERATING UNITS (if any)  
None

LAB/BRANCH  
Laboratory of Vision Research

SECTION  
Section on Retinal and Corneal Metabolism

INSTITUTE AND LOCATION  
National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 1.2	PROFESSIONAL: 0.6	OTHER: 0.6
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CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS      ☐ (b) HUMAN TISSUES      ☒ (c) NEITHER

☐ (a1) MINORS    ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Bovine and frog rod outer segment preparations catalyze the transfer of N-acetylgalactosamine to rhodopsin. This sugar is not found in rhodopsin as it is ordinarily isolated and purified but may represent a transient component found only in the plasma membrane rhodopsin. As such, it could serve as a specific marker to mediate cell surface interactions such as the phagocytosis of shed outer segment tips by the pigment epithelium.

Project Description:

Objectives: Many interactions between macromolecules and cell membranes are mediated by the sugar molecules bound to one of the interacting surfaces. In the process of renewal of photoreceptor outer segment disc membranes, rhodopsin, a glycoprotein, must be transported from the inner segment and incorporated into disc membranes with a specific orientation in space. This project was designed to determine where and when sugars are added to the polypeptide and what role they play in the transport and assembly of rhodopsin into disc membranes and in the process of shedding and phagocytosis of disc membranes.

Methods Employed: Ordinary biochemical techniques were used, such as cell-fractionation, isolation of rod outer segments by density gradient centrifugation, detergent extraction and purification of rhodopsin by column chromatography, and incubation of outer segment preparations.

Major Findings: A new component of rhodopsin, N-acetylgalactosamine, has been transferred from the sugar nucleotide to rhodopsin using both bovine and frog rod outer segment preparations. The transfer of this sugar as well as galactose and fucose appears to involve plasma membranes of the outer segment rather than disc membranes.

Significance to Biomedical Research and the Program of the Institute: There are now three sugars, not ordinarily found in purified rhodopsin, that can be incorporated into rhodopsin by outer segment preparations: galactose, fucose, and N-acetylgalactosamine. These three sugars appear to be associated with the rhodopsin in the plasma membrane and may provide a unique marker to mediate cell surface interactions. The addition or removal of such unique structures could provide the pigment epithelium with a mechanism to distinguish between intact outer segments and shed tips which must be phagocytized.

Proposed Course: The presence of these enzyme activities will be determined in animals with inherited retinal degenerations in the hope of finding specific metabolic lesions. Possible intermediates in the transfer reactions such as retinyl phosphate sugars will also be sought as will the role of the pigment epithelium in controlling these reactions.

NEI Research Program: Retinal and Choroidal Diseases--Visual Cells and Pigment Epithelium

Publications:

O'Brien PJ: Characteristics of galactosyl and fucosyl transfer to bovine rhodopsin. Exp Eye Res 26:197-206, 1978.

Tamai M, Teirstein R, Goldman A, O'Brien P, Chader GJ: The pineal gland does not control rod outer segment shedding and phagocytosis in the rat retina and pigment epithelium. Invest Ophthalmol Visual Sci 17:558-562, 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00024-04 LVR
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PERIOD COVERED  
October 1, 1977 to September 30, 1978

TITLE OF PROJECT (80 characters or less)  
Intermediary Metabolism of the Cornea

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David R. Whikehart	Ph.D.	Senior Staff Fellow	LVR	NEI
Other:	R. Theodore Fletcher	M.S.	Chemist	LVR	NEI

COOPERATING UNITS (if any)  
None

LAB/BRANCH  
Laboratory of Vision Research

SECTION  
Section on Retinal and Corneal Metabolism

INSTITUTE AND LOCATION  
National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS: 1.0	PROFESSIONAL: 0.8	OTHER: 0.2
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CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS      ☐ (b) HUMAN TISSUES      ☒ (c) NEITHER

☐ (a1) MINORS    ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This investigation concerns itself with the biochemical mechanisms that control corneal hydration (deturgescence). Tissue cultures from rabbit corneal endothelia have been grown in order to compare their biochemistry with that of the fresh tissue cells. Cyclic AMP and cyclic GMP have been measured and compared in the fresh tissues and the tissue cultures of primary outgrowths and subcultured cells approaching confluency. It was shown that these cyclic nucleotides, which control a number of cell functions, were quite highly concentrated in these cells, were similarly concentrated in fresh tissues and subcultures, and were somewhat less concentrated in primary outgrowths.

Project Description:

Objectives: This project has been initiated to elucidate the biochemistry of corneal deturgescence and to suggest mechanisms for its control. Its immediate objectives are: 1) to determine the location, accessibility and reactivity of specific areas of the plasma membrane of the corneal endothelium to cytoplasmic, sulfhydryl/disulfide metabolites, 2) to measure intracellular levels of adenosine, 3) to establish better conditions for tissue culture and/or eye bank storage by observing what metabolites or activators produce optimal ATPase activity.

Methods Employed: Major sources of tissue have been rabbit and bovine eyes. Extremely sensitive and accurate techniques of assay employing double-beam spectrophotometry and gas liquid chromatography have been employed. These procedures assay intermediates at the nanogram level. Tissue cultures of endothelial cells have been initiated from micro-dissected endothelial/Descemet's layer buttons from rabbits. Perfusions of rabbit corneas have been accomplished with the aid of the specular microscope to measure corneal swelling rates. Cyclic nucleotides have been assayed by radioimmunoassay.

Major Findings: Quantities of adenosine 3',5'-cyclic monophosphate and guanosine 3',5'-cyclic monophosphate were measured in rabbit corneal endothelial cells taken from fresh tissue and tissue culture. Higher levels of both nucleotides were observed when the cells were initially frozen in liquid nitrogen compared with those that were not. Adenosine 3',5'-cyclic monophosphate levels were 14 times lower in the primary cultures compared to the fresh tissue while subcultures had about two-thirds the amount of fresh tissue. Guanosine 3',5'-cyclic monophosphate was four times more concentrated in the primary cultures than in fresh tissue while levels in subcultures were about two-thirds those of the fresh tissue. The ratios of adenosine 3',5'-cyclic monophosphate to guanosine 3',5'-cyclic monophosphate were high in both fresh tissue and subcultured cells approaching confluency, but low in primary outgrowths.

Significance to Biomedical Research and the Program of the Institute: Corneal diseases involving disturbances in the proper hydration (deturgescence) of the cornea (resulting in cloudy, impaired vision) are thought to be the result of metabolic dysfunction (degeneration). Such dysfunctions are the result of damage from transplants, storage conditions (cornea banks), inflammatory reactions and inherent dystrophies. Presently, however, the normal metabolic functions associated with the hydration pump(s) and its controls have not been adequately described. The investigation of the cyclic nucleotides in the corneal endothelial cells are important since these nucleotides (cAMP and cGMP) have been observed to control growth and numerous other cell functions in a host of other cell types. The high levels of these nucleotides in the endothelia may suggest their importance in the operation of the deturgescent mechanism and implicate a possible role for them in the control of significant ATPases in the cell membrane.

Proposed Course: The present project has been terminated.

NEI Reserach Program: Corneal Diseases--Corneal Edema, Dystrophies,  
and Inherited Disorders

Publications:

Whikehart DR, Edelhauser HF: Glutathione in rabbit corneal endothelia:  
The effects of selected perfusion fluids. Invest Ophthalmol Visual Sci  
17:455-464, 1978.

Masterson E, Whikehart DR, Chader GJ: Glucose oxidation in the chick  
cornea: Effect of diamide on the pentose shunt. Invest Ophthalmol Visual  
Sci 17:449-454, 1978.

Whikehart DR: Glutathione peroxidase activity in the bovine corneal  
endothelium: A comparison with its activity in the corneal epithelium  
and whole lens. Ophthalmic Res (in press).

Whikehart DR: Oxidized glutathione in the cornea: A note on technique.  
Exp Eye Res (in press).



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00070-01 LVR																				
PERIOD COVERED October 1, 1977 to September 30, 1978																						
TITLE OF PROJECT (80 characters or less)  Vitamin A and Ocular Tissues																						
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 30%;">Barbara Wiggert</td> <td style="width: 15%;">Ph.D.</td> <td style="width: 20%;">Staff Fellow</td> <td style="width: 20%;">LVR NEI</td> </tr> <tr> <td>Other:</td> <td>Julia Derr</td> <td>B.A.</td> <td>Biologist</td> <td>LVR NEI</td> </tr> <tr> <td></td> <td>Paul Russell</td> <td>Ph.D.</td> <td>Staff Fellow</td> <td>LVR NEI</td> </tr> <tr> <td></td> <td>Gerald J. Chader</td> <td>Ph.D.</td> <td>Research Chemist</td> <td>LVR NEI</td> </tr> </table>			PI:	Barbara Wiggert	Ph.D.	Staff Fellow	LVR NEI	Other:	Julia Derr	B.A.	Biologist	LVR NEI		Paul Russell	Ph.D.	Staff Fellow	LVR NEI		Gerald J. Chader	Ph.D.	Research Chemist	LVR NEI
PI:	Barbara Wiggert	Ph.D.	Staff Fellow	LVR NEI																		
Other:	Julia Derr	B.A.	Biologist	LVR NEI																		
	Paul Russell	Ph.D.	Staff Fellow	LVR NEI																		
	Gerald J. Chader	Ph.D.	Research Chemist	LVR NEI																		
COOPERATING UNITS (if any)  None																						
LAB/BRANCH Laboratory of Vision Research																						
SECTION Section on Retinal and Corneal Metabolism																						
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20014																						
TOTAL MANYEARS: 1.9	PROFESSIONAL: 1.4	OTHER: 0.5																				
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																						
SUMMARY OF WORK (200 words or less - underline keywords)  Specific binding proteins (" <u>receptors</u> ") for <u>retinoids</u> are present in normal retina, pigment epithelium, choroid, and cornea. Receptors are also present in cultured chick pigment epithelial cells and in Y-79 <u>human retinoblastoma cells</u> grown in tissue culture. The retinoid receptors appear to transport and mediate the effects of <u>vitamin A</u> in both normal and diseased ocular tissues.																						

Project Description:

Objectives: To elucidate the mechanism of action of retinoids in ocular tissues.

Methods Employed: Retinoid receptors in cytosol samples were determined by sucrose density gradient centrifugation, slab gel electrophoresis, and iso-electric focusing.

Major Findings:

1) The cytosol fraction of human retinoblastoma cells grown in tissue culture contains separate retinol and retinoic acid receptors. After retinoic acid is bound to its receptor in the cytosol, the receptor-retinoic acid complex is apparently translocated to the cell nucleus. Thus, retinoic acid may play a role similar to that of steroid hormones in controlling cellular differentiation. The retinol receptor appears to remain in the cytosol where it may affect glycoprotein biosynthesis.

2) In chick pigment epithelial cells grown in tissue culture, there is a cytosol receptor for retinol but not for retinoic acid. Similarly, in developing and hatched chick pigment epithelial cytosol, there is a receptor for retinol but not for retinoic acid. Chick choroidal cytosol has both a retinol and a retinoic acid receptor. Bovine corneal epithelium, stroma, and endothelium each contain a cytosol receptor for retinol. Corneal endothelium, but not corneal epithelium, contains a receptor for retinoic acid. The absence of a retinoic acid receptor in some tissues indicates the possibility of important differences in the use of retinoids by different tissue layers in the eye.

3) Binding of retinol to a 7S receptor which is apparently compartmentalized in retinal rod outer segments is significantly lower in the soluble fraction of dark-adapted as compared with light-adapted bovine and frog eyes. This light-dark difference in retinol binding may indicate an important role for the 7S retinol receptor in the visual process, possibly as a transport protein for retinol.

Significance to Biomedical Research and the Program of the Institute:

It is hoped that a better understanding of the mechanism of action of retinoids in maintaining normal cell function will contribute to the prevention of diseases such as keratomalacia and retinal degeneration where vitamin A may be involved. In addition, since retinoid receptors are present in human retinoblastoma cells, it is possible that retinoids may one day be of value in the chemoprevention or chemotherapy of this tumor.

Proposed Course: The role of specific receptors in mediating the action of retinoids in ocular tissues will continue to be investigated in both fresh tissue and in cells grown in tissue culture.

NEI Research Program: Retinal and Choroidal Diseases--Developmental and Hereditary Disorders

Publications:

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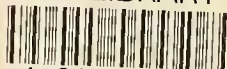
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